Blood Conservation in Cardiac Surgery

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Declaration

I **Robert David Slight** declare that;

1. This thesis has been composed by myself
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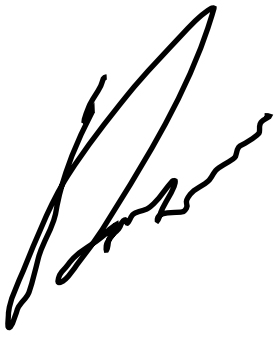
Signed: 

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I would like to dedicate this thesis to my fiancee family and friends....

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Publications

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Abstract

Cardiac surgery is traditionally a heavy user of blood and blood products. Until recently, the benefits of transfusion have been largely assumed and the risks relatively ignored. This has prompted us to examine new ways of minimising patient exposure to donor red blood cells (RBC's). At the present time, most clinical guidelines for RBC transfusion are based mainly upon haemoglobin concentration ([Hb]). As [Hb] may be artificially depressed by the haemodiluting effect of the heavy clear fluid load associated with cardiac surgery, transfusing based upon [Hb] alone may overestimate the requirement for RBC's. Where such haemodilution is present, systemic oxygenation may be maintained through a viscosity mediated patho-physiological response. The work reported in this thesis attempts to explore the relative contribution of both red cell volume (RCV) and plasma volume (PV) to the anaemia encountered following cardiac surgery while also examining factors that may be associated with a low post-operative RCV. In addition, we have explored on a theoretical basis what [Hb] would represent a critical level of systemic oxygen delivery (DO_{2Crit}). Taken together, this has allowed us to develop an RCV based transfusion guideline aimed at reducing the incidence of unnecessary (and potentially counter-productive) RBC transfusion. As RBC's may be associated with pulmonary endothelial damage, we have also studied the impact of the RCV guideline developed on post-operative acute lung injury (ALI). Finally, in a separate study, the merits of a simple activated clotting time (ACT) based system of anti-coagulation management for cardiopulmonary bypass (CPB) versus that of an individualised heparin management system (HMS) are described.

Acronyms

[HCO3$^-$]	bicarbonate ion.
[Hb$_{Crit}$]	critical haemoglobin concentration.
[Hb]	haemoglobin concentration.
2,3-DPG	2,3-diphosphoglycerate.
ACC	aortic cross clamp.
ACS	acute coronary syndrome.
ACT	activated clotting time.
ACT$_{Grp}$	activated clotting time management group.
AHTR	acute haemolytic transfusion reaction.
ALI	acute lung injury.
APTTr	activated partial thromboplastin time ratio.
ASD	atrial septal defect.
AT-III	anti-thrombin III.
ATR	acute transfusion reaction.
AVR	aortic valve replacement.
BFI	blood flow index.
BH$_{Conc}$	blood heparin concentration.
BIA	bio-electrical impedance analysis.
BSA	body surface area.
BSE	bovine spongiform encephalopathy.
BV	blood volume.
CABG	coronary artery bypass grafting.
CAD	coronary artery disease.
CaO_2	arterial oxygen content.
CI	cardiac index.
CJD	Creutzfeldt-Jakob disease.
CM	carbon monoxide.

CO	cardiac output.
COAD	chronic obstructive airways disease.
CPB	cardio-pulmonary bypass.
CVA	cerebro-vascular accident.
CVD	cerebro-vascular disease.
CvO_2	venous oxygen content.
CXR	chest x-ray.
DAT	direct antiglobulin testing.
DHTR	delayed haemolytic transfusion reaction.
DIC	disseminated intravascular coagulation.
DNR	do not resuscitate.
DO_2	oxygen delivery.
DO_{2Crit}	critical oxygen delivery.
DoH	Department of Health.
DTR	delayed transfusion reaction.
ECF	extra-cellular fluid.
ECG	electro-cardiogram.
FEV1	forced expiratory volume 1s.
FFM	fat free mass.
FFP	fresh frozen plasma.
FNHTR	febrile non-haemolytic transfusion reaction.
FVC	forced vital capacity.
Hb_{Grp}	haemoglobin threshold transfusion group.
Hct	haematocrit.
HDR	heparin dose responsiveness.
HIV	human immunodeficiency virus.
HLA	human leucocyte antigen.
HMS	heparin management system.
HMS_{Grp}	heparin management system group.
HPT	heparin protamine titration.
IABP	intra-aortic balloon pump.
ICSH	International Committee on Standardisation in Haematology.
ICU	intensive care unit.
ICU_{Ret}	return to the intensive care unit.
ISBT	International Society for blood Transfusion.

IU	International Units.
LMS	left main stem.
LREC	Lothian Regional Ethics Committee.
MAP	mean arterial pressure.
MHRA	Medicines and Healthcare Regulatory Agency.
MI	myocardial infarction.
MVR	mitral valve repair/replacement.
NaF	sodium fluorescein.
NBTS	National Blood Transfusion Service.
NHS	National Health Service.
NO	nitric oxide.
$NoRBC_{Grp}$	no red blood cell transfusion group.
NYHA	New York Heart Association.
P_{Val}	P value.
$PaCO_2$	arterial partial pressure of carbon dioxide.
PaO_2	arterial partial pressure of oxygen.
PDH	pyruvate dehydrogenase.
PFT	pulmonary function test.
PH_{Conc}	plasma heparin concentration.
PIHD	pre-operative isovolaemic haemodilution.
Plt	platelet count.
ppb	parts per billion.
PTP	post-transfusion purpura.
PTr	pro-thrombin time ratio.
PV	plasma volume.
PVD	peripheral vascular disease.
PVO_2	mixed venous oxygen partial pressure.
PVR	pulmonary vascular resistance.
QO_2	oxygen extraction.
QO_{2Crit}	critical oxygen extraction.
QOL	quality of life.
r	correlation coefficient.
RBC	red blood cell.
RBC_{Grp}	red blood cell transfusion group.
RCT	randomised controlled trial.

RCV	red cell volume.
RCV_{Grp}	red cell volume guideline transfusion group.
SABRE	serious adverse blood reactions and events.
SaO_2	arterial oxygen saturation.
SASM	Scottish Audit of Surgical Mortality.
SBE	standard base excess.
SD	standard deviation.
SEM	standard error of the mean.
SHOT	serious hazards of transfusion.
SIGN	Scottish Intercollegiate Guideline Network.
$SJvO_2$	jugular venous oxygen saturation.
SNBTS	Scottish National Blood Transfusion Service.
SvO_2	mixed venous oxygen saturation.
TA-GVHD	transfusion associated graft versus host disease.
TACO	transfusion associated circulatory overload.
TAT	thrombin anti-thrombin.
TBW	total body water.
TRALI	transfusion related acute lung injury.
TRIM	transfusion related immunodilution.
UPPO	units per patient operated.
UPPT	units per patient transfused.
USP	United States Pharmacopeia.
vCJD	variant Creutzfeldt-Jakob disease.
VO_2	oxygen consumption.
WBC	white blood cell.
ZO_2	solubility coefficient for oxygen in blood.

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Part I

Background and Programme of Research

Chapter 1

Introduction

1.1 Overview

The first half of the twentieth century saw the evolution of techniques in blood preservation and banking that permitted the beginnings of today's liberal transfusion practice[1]. The benefits of transfusion have always been assumed, the risks largely ignored until the association between transfusion and viral hepatitis became more widely recognised in the late 1960's[2]. Since, the list of complications has grown including the recent recognition of transmission of variant Creutzfeldt-Jakob disease (vCJD) by blood from persons who inter-developed vCJD[3].

Cardiac surgery remains a heavy user of blood and blood products[4]. In part, this may be explained by improvements in clinical care which have led to more complex procedures being performed on an increasingly elderly population[5].

Recently, attention has been drawn to the general intensive care unit (ICU) with regard to the efficacy of red blood cell (RBC) transfusion. In this population, the only large and well conducted randomised controlled trial (RCT) showed that a liberal RBC transfusion policy may be counter-productive[6]. Thus a timely re-appraisal of the role of RBC transfusion in cardiac surgery is required.

In the following sections we consider;

- A brief history of the development and use of autologous blood (sections 1.2 and 1.3).
- The risks associated with blood transfusion (section 1.4).
- Factors that may be relevant in deciding whether or not to administer a blood transfusion (section 1.5).

1.2 The History and Development of Blood Transfusion

1.2.1 Early Beginnings

RBC transfusion has given itself a reputation as a simple and life saving procedure since the turn of the twentieth century[7;8]. The foundations of this technique were laid by the ancient Greeks and Romans circa 430 years BC. The first description of blood as an essential basic ingredient of man was given by Hippocrates in his central doctrine 'On the nature of man'. He suggested that the four foundation blocks of man were blood, phlegm, yellow bile and black bile. It was considered that any illness was attributable to an imbalance between these humors and that attention to diet and environment would result in their restoration[9–11].

Despite this early recognition of the role of blood, further advances were limited by an inadequate knowledge of human circulatory physiology and anatomy. It was William Harvey who first described accurately the human circulation in his work of 1628 entitled "Exercitatio anatomica de motu cordis et sanguines in animalibus". This was followed by the first experiments with animal-animal transfusion in Oxford around 1666 by Richard Lower. The first animal-human transfusion was carried out by Jean Denis of Paris in 1667. There followed a pause prior to the first human-human transfusion[9–12].

Obstetrician James Blundell of Guy's and St Thomas's Hospital in London observed many cases of fatal post-partum haemorrhage. He demonstrated that death from haemorrhage in dogs could be prevented by re-infusing shed blood. This discovery was somewhat tempered by his finding that nearly all dogs died when given human blood. However, the direct result was his recommendation that humans should only be given human blood. This he carried out successfully with the aid of self designed transfusion apparatus. The net result was that survival following post-partum haemorrhage was greatly enhanced. This began the rapid development of transfusion medicine some 2000 years after the Hippocrates first described the humoral elements (figure 1.1)[9–12].

Figure (1.1) *Painting of an early blood transfusion. Reproduced from the Canadian library archives (http://www.collectionscanada.ca).*

1.2.2 Blood Grouping

As mentioned above, it was recognised that differences in the inter-species compatibility of blood could lead to cell lysis and ultimately death. However, blood variability within man remained undescribed until 1901. Karl Landsteiner based his description on experiments with blood and serum interactions in several human subjects. He noted that certain combinations of blood and serum would result in red cell clumping and postulated the existence of an immunological mechanism. Initially, three blood groups were described (A, B and C) based on his observations[7;13]. Decastello & Sturli further expanded on this work the following year and identified a fourth blood group (AB). It was also recognised that the so called "agglutinins" present were not associated with disease. Several other groups duplicated Landsteiner's work and different nomenclature's for grouping systems were introduced. This was resolved in 1937 by the International Society for blood Transfusion (ISBT) when today's ABO nomenclature was adopted as

the universal standard. The discovery of the major human blood group system was paralleled by the development of methods that would allow the storage and preservation of donated blood[7;14].

1.2.3 Red Cell Storage

In order to provide adequate storage of RBCs a medium that both anti-coagulates and preserves is required. Hammarstein in 1875 was the first to describe the dramatic effects of the calcium salts on the speed of blood clotting. Griesbach (1891) noted that the addition of ammonium citrate to blood would cause it to remain in a fluid state. It was subsequently realised this related primarily to the affinity of calcium for citric acid. Other anti-coagulants (oxalates, tartrates) had been described, but citrate was the only one that did not cause precipitation of calcium salts. Sabbatini effectively demonstrated this property in 1902 and went on to describe how citrate reduced the concentration of ionised calcium below the concentration required for blood clotting[7;9;15].

The first transfusion of citrated blood in a human is accredited to Hustin in 1914. After first experimenting on animals he concluded that the addition of 0.2% citrate could delay blood clotting for as long as thirty minutes. He also noted that RBCs in citrate did not survive as well as those in saline. Thus, the citrate was in part replaced by glucose prior to the first human administration. This realisation was further investigated by Rous & Turner in 1916. They experimented with several sugar solutions aimed at minimising RBC lysis on storage. Ultimately it was concluded that the storage of three parts human blood with two parts 3.8% citrate and five parts of 5.4% glucose would result in the adequate storage of RBCs for up to a month[15;16]. It was mistakenly thought at this time that RBCs were impervious to sugars and that it was their colloidal properties that produced the preservative effect. The discovery was made later of the role of glucose in RBC anaerobic metabolism. As the so called Rous & Turner solution required a degree of sedimentation in order to remove excess supernatant, further refinements were made resulting in a more compact storage medium by the time of the Second World War[1;17].

1.2.4 Blood Banking

The first recorded storage of blood in advance of clinical requirement was carried out by Oswald Robertson in 1918 behind the front line in France[18]. Donors selected were healthy young men with trivial wounds sustained on the field of battle. The average storage time was 10-14 days prior to transfusion. Recipients were those critically injured but thought to have a chance of survival if given a transfusion. In total 22 transfusions were given to 20 individuals. 11 out of the 20 individuals transfused were fit enough to be transferred back for active duty after recovering from their injuries. Furthermore, it was noted that the immediate improvement observed was just as prevalent with blood stored for up to 3-4 weeks as with that stored for shorter periods[17]. Robertson concluded that the principle priority with his technique was to have sufficient stored blood to cope with any sudden increase in demand. The basic principles of modern blood banking had been established[17;18].

The first actual blood donor service was established by Percy Oliver (1921) in London. Oliver's division of the Red Cross received an appeal from Kings College Hospital for blood donors. Members dutifully gave blood and Oliver was prompted to set up a list of family and friends who could be called at short notice to donate blood. Donors gave their services free of charge although would be re-reimbursed travel expenses. Although there was a slow uptake, word spread and Oliver's services were called upon 428 times in 1925[9;14]. At this time many doctors remained reluctant to use a citrated anti-coagulant storage solution and much reliance was placed upon direct transfusion. In addition, many people died from ABO incompatibility as typing techniques were unreliable. Despite this, donor lists sprang up in major cities around the UK and the practice became adopted worldwide[7;9;19].

Frederico Duran-Jordan, a physician during the Spanish civil war, is credited with the first modern system of blood banking. A mobile storage unit of group O blood was held and transported wherever required (figure 1.2)[14;20]. Following the war he fled to London where he helped establish a similar system. At that time further conflict with Germany was imminent and the war office established the army blood supply depot. This was again a mobile system that held blood

Figure (1.2) *Mobile blood bank during the Spanish civil war. Reproduced from the Canadian library archives (http://www.collectionscanada.ca).*

centrally and transported it forward wherever required. By the end of the second world war war 1300 units of blood were supplied and used each day. Following the success of this system, and the creation of the National Health Service (NHS), both the National Blood Transfusion Service (NBTS) and Scottish National Blood Transfusion Service (SNBTS) were established in 1946[17;19;21].

1.2.5 Where Did We Get a 'Transfusion Threshold'?

The first mention of a haemoglobin concentration ([Hb]) based transfusion threshold can be traced back to 1941. Lundy wrote, without providing reference to any supporting evidence, with respect to anaemia "This condition, owing to the lowered oxygen carrying capacity of the blood, interferes with the adequate transportation of oxygen to the tissues. When the concentration of haemoglobin is less than 8-10g per 100cc of whole blood it is wise to give a blood transfusion before operation"[22;23]. All though this statement could be deemed unfounded on the basis of scientific or clinical evidence available at the time, subsequent attempts were made at its justification[24]. However, the mathematical demonstrations of

poor oxygen kinetics, when [Hb] is reduced, that followed were extremely limited as no consideration was given to the effects of general anaesthesia on basal metabolic rate nor the scope for haemodynamic compensation in terms of an increase in cardiac output (CO). Furthermore, as this was prior to the advent of hypothermic cardio-pulmonary bypass (CPB), no consideration was given to temperature, a variable particularly applicable to cardiac surgery[25].

Interestingly, although over half a century has passed since Lundy's unsubstantiated statement, RBCs are still widely administered based upon the [Hb] level originally proposed by Lundy as a prudent threshold for transfusing RBCs.

1.3 Current Blood Usage

Blood transfusion therapy has become an everyday component of medical management since its early development as outlined above. At present, precise UK wide information on blood usage does not exist[26;27].

A retrospective analysis conducted during 2000/2001 estimated a total of 2.8 million whole blood donations were collected in the UK. This accounted for a total of 1.7 million transfusion events at a total cost of 898 million pounds in real terms. RBCs accounted for 0.98 million transfusion events averaging 2.7u per transfusion[28]. When compared to a previous study by the same group in 1994/1995, it was estimated transfusion costs had risen by 256%[29]. Attempts have been made to establish exactly where all this blood is used.

A prospective study in the North of England recorded the destination of 9774u of RBCs over two fourteen day periods in 1999 and 2000. Overall, 40.7% of units were used by surgical departments, the commonest indications being total hip replacement (4.6%) and coronary artery bypass grafting (CABG) (4.1%)[26]. Within the Edinburgh cardiac surgery unit, RBC transfusion practice was studied in 1997. A cohort of 74 elective patients were recorded over a 1 month period. Overall, 85% of patients were given RBCs. Those transfused on average received 2.3u with a figure of 2.1u per patient operated upon[30]. The Safe And Good Use of Blood Products in Surgery (SANGUIS) study recorded transfusion rates between 17.1 - 100% for CABG procedures across European units in 1989-90. At that time 93% of CABG procedures in Edinburgh were transfused[31]. It should be

added however, that the transfusion strategy in Edinburgh is now considerably more restrictive than the strategy employed in 1989-90.

1.4 The Risks and Monitoring of Transfusion

The potential risks and complications of any RBC transfusion are numerous and the true incidence of adverse events and reactions is almost certainly only partially documented. In the following sections we firstly describe the UK haemovigilance schemes followed by the specific risks of transfusing RBCs. Finally, we examine the impact of RBC transfusion on mortality and long term outcome.

1.4.1 Haemovigilance in the UK

The recognition that the monitoring of transfusion complications is essential for developing risk management strategies has led to the development of haemovigilance schemes in many countries[32;33].Haemovigilance may be defined as 'a set of surveillance procedures covering the entire transfusion chain (fromthe donation of blood and its components to the follow-up of recipients of transfusions), intended to collect and assess information on unexpected or undesirable effects resulting from the therapeutic use of labile blood products, and to prevent the occurrence or recurrence of such incidents'[34].

In the UK, haemovigilance of adverse events and reactions has been carried out by the serious hazards of transfusion (SHOT) scheme. Since its implementation in 1996, this system has relied upon the voluntarily return of questionnaires documenting adverse transfusion events and 'near misses'[35]. Only recently has the reporting of adverse events become compulsory. The recent EU 'blood safety directive' introduced a legal requirement for the reporting of adverse events in member states to a competent authority. This was subsequently enforced in the UK by the Department of Healths (DoHs) 'Better Blood' initiative and the enactment of the UK 'Blood Safety (and quality) Regulations 2005'. The Medicines and Healthcare Regulatory Agency (MHRA) was appointed as the competent reporting authority with the serious adverse blood reactions and events (SABRE)

online reporting system subsequently being put into place[36]. A summary and description of reportable SHOT events is given in table (table 1.1)[35].

1.4.2 Specific Complications of Transfusion

In the following sections we discuss the pathogenesis, diagnosis and management of the principle specific complications of RBC transfusion. A summary table of events reported to SHOT 1996 - 2004 is given in table 1.2[33;37].

1.4.2.1 Acute Transfusion Reactions

Acute transfusion reactions (ATRs) include;

- Febrile non-haemolytic transfusion reactions
- Acute haemolytic transfusion reactions
- Reaction to bacterially contaminated blood
- Allergic reaction or anaphylaxis
- Transfusion related acute lung injury
- Transfusion associated circulatory overload

Transfusion related acute lung injury (TRALI) and transfusion associated circulatory overload (TACO) are discussed in more detail in sections 1.4.2.6 and 1.4.2.7 respectively.

Febrile Non-haemolytic Transfusion Reactions

Febrile non-haemolytic transfusion reactions (FNHTRs) are not reportable to SHOT but are particularly frequent. Such reactions are characterised by fever, nausea, dyspnoea and occasionally hypotension. Onset is usually during or shortly after the RBC transfusion has taken place[35;38;39]. The primary mechanism for these reactions is thought to be the interaction between the recipient's cytotoxic antibodies and donor leucocytes, leading to the release of endogenous pyrogens[40]. The incidence of these reactions has reduced by as much as 50%

with universal leucodepletion. Preventative measures include the administration of anti-histamines, anti-pyretics such as paracetamol or corticosteroids[41;42].

Acute Haemolytic Transfusion Reaction

Acute haemolytic transfusion reactions (AHTRs) may occur in a number of ways. One of the commonest causes is the transfusion of ABO incompatible blood, usually as a consequence of human error[42]. Other causes of haemolysis include the chemical or mechanical destruction of RBCs. Lysis may result from the use of hypotonic fluids, overheating or improper freezing of units and prolonged storage. Mechanical destruction can occur by the use of pumps or small cannulae for transfusion[38]. Symptoms can be severe, depending on the degree of haemolysis, including hypotension, shock and renal failure. Diagnosis can be confirmed with the presence of free plasma haemoglobin and haemoglobinuria. Treatment is supportive with the maintenance of urine output by a diuretic where required. Severe cases may require inotropic support and renal filtration[38;39;42].

Reaction to Bacterially Contaminated Blood

Most cases of bacterial infection occur with platelet transfusion, primarily because the product is stored at room temperature rather than the 2-4^{0}C of RBCs. Organisms commonly implicated include Staphylococcus, Yersinia, Enterobacter and Pseudomonas[43;44]. Potential donors with risk factors for blood borne bacterial infection are excluded from blood donation. However, this does not prevent asymptomatic donors with a transient bacteremia from giving blood. No screening tests are currently available to detect contaminated units. Recently, platelet samples in Scotland have started being withdrawn for culture although this is not a common practice[44]. Typical presentation is with sepsis and circulatory shock in a matter of minutes or hours after the completion of transfusion[35;39;43]. Blood samples should be taken from the patient and the suspected unit for culture. Treatment is with broad-spectrum antibiotic therapy and cardio-respiratory support where required[43].

Table (1.1) *Reportable UK haemovigilance (SHOT) events. SHOT = Serious Hazards of Transfusion and HPA = human platelet antigen . Reproduced from SHOT report 2005 (http://www.shotuk.org).*

Event	SHOT Definition
Incorrect blood component transfused	Patient transfused with a blood component or product which did not meet the appropriate requirement or was intended for another patient
Acute transfusion reaction	Adverse reactions occurring up to 24h following transfusion, excluding those due to incompatible blood
Delayed transfusion reaction	Clinical adverse reactions (not simple serological reactions) occurring more than 24h following transfusion of blood components
Transfusion related acute lung injury	Acute dyspnoea with hypoxia and pulmonary infiltrates within 24h of transfusion, with no other apparent cause
Transfusion-associated graft versus host disease	Development of the classical symptoms of fever, rash, liver dysfunction and pancytopenia occurring 16 weeks post-transfusion
Post-transfusion purpura	Thrombocytopenia 512 days post-transfusion associated with antibodies in the patient directed against the HPA system
Transfusion-transmitted infection	Post-transfusion infection in which the recipient had no evidence of infection pre-transfusion where at least one component was was shown to have been contaminated with the infective agent
"Near-miss "event	Any error which, if undetected, could result in the determination of a wrong blood group, or issue, collection or administration of an incorrect, inappropriate or unsuitable component but which was recognised before transfusion took place

Table (1.2) *Summary table of SHOT Data 1996 - 2004. SHOT = Serious Hazards of Transfusion, IBCT = incompatible blood component transfused, ATR = acute transfusion reaction, DTR = delayed transfusion reaction, PTP = post-transfusion purpura, TA-GVHD = transfusion associated graft versus host disease, TRALI = transfusion related acute lung injury and TTI = transfusion transmitted infection. Reproduced from SHOT reports 1996 - 2004 (http://www.shotuk.org).*

	IBCT	ATR	DTR	PTP	TA-GVHD	TRALI	TTI	**Total**
Cause of Death	6	2	6	1	13	8	9	45
Probably Caused Death	3	3	1	0	0	5	0	12
Possibly Caused Death	11	7	1	1	0	23	0	43
Severe Morbidity	92	6	28	13	0	93	36	268
Minor Morbidity	1709	246	219	29	0	33	4	2240
Unknown Outcome	11	3	1	0	0	0	0	15
Total	**1832**	**267**	**256**	**44**	**13**	**162**	**49**	**2623**

Allergic Reaction or Anaphylaxis

Allergic reactions following transfusion are common but fortunately usually mild. Typically symptoms are cutaneous with pruritis accompanied by both erythematous and papular rashes. These reactions are usually IgE mediated with symptoms due to histamine release. Treatment consists of interrupting the transfusion and the administration of an anti-histamine. Providing fever is not present the transfusion may be recommenced following this action. At the other end of the clinical spectrum is severe, potentially lethal, anaphylaxis. Patients may develop laryngeal oedema with hypotensive shock. Management should be prompt with adrenaline, vasopressor agents and airway management where required[38;39;42;45]. Leucodepletion does not prevent allergic or anaphylactic reactions as they are thought to be triggered by soluble substances in donor plasma[46].

1.4.2.2 Delayed Transfusion Reactions

Delayed transfusion reactions (DTRs) include;

- Delayed haemolytic transfusion reaction
- Post-transfusion purpura
- Transfusion associated graft versus host disease

Delayed Haemolytic Transfusion Reaction

Delayed haemolytic transfusion reactions (DHTRs) describe haemolysis that typically occurs between 4 - 10 days following the offending transfusion. Patients have usually been pre-sensitised to a RBC antigen either by child birth or previous transfusion[42]. Clinical features include a gradually falling [Hb], jaundice and occasionally haemoglobinuria or renal failure[47]. The diagnosis can be confirmed by the use of direct antiglobulin testing (DAT). Usually, the responsible antibody is present on initial screening but at levels below clinical detection. Rarely, the patient may have no pre-transfusion antibody but develops one while the sensitising RBCs are in the circulation. Risk is minimised in most centres by the provision of antigen matched RBCs[42]. Treatment is rarely required aside from

the careful monitoring of renal function[39].

Post-transfusion Purpura

Post-transfusion purpura (PTP) is a rare bleeding disorder characterised by an acute thrombocytopenia occurring 5-10 days following RBC transfusion. The condition is often missed because of the delay between the offending transfusion and the onset of symptoms[39;48;49]. The cause is thought to be platelet alloimmunisation in pre-sensitised individuals such as multiparous females, although the precise mechanism is still controversial. Diagnosis of PTP can be confirmed by the presence of anti-platelet antibodies in the recipient[49;50]. The incidence of PTP has fallen dramatically in this country since the introduction of universal leucodepletion. Primarily, this is thought to relate to the removal of platelets from RBCs as part of the above process[41]. Treatment consists of the administration of high dose intra-venous immunoglobulin, with or without steroids[39;48;49].

Transfusion Associated Graft Versus Host Disease

Transfusion associated graft versus host disease (TA-GVHD) is a rare complication of transfusion which is fatal in virtually all cases. The risk of TA-GVHD is related to the number of T-lymphocytes transfused, the recipient's immune status and the relationship between donor and recipient human leucocyte antigen (HLA) types[51–53]. Symptoms usually consist of rash, fever, abdominal pain, liver dysfunction and bone marrow suppression some 2-30 days following transfusion[39]. Death usually occurs due to infection. Treatment is essentially preventative with blood products for at risk groups being irradiated. Immunosuppressive therapy has been used for those who contract the condition albeit with limited results[51;52]. Leucodepletion has again had a profound effect on the incidence of the disease[41].

1.4.2.3 Transfusion Related Immunodilution

Any blood product administered may contain a large amount of foreign antigenic material in both soluble and cell associated forms. Such alloantigenic matter may result in a down regulation of the host immune response. Commonly, this series of

events is referred to as transfusion related immunodilution (TRIM)[54;55]. In surgical patients, such a scenario is generally considered deleterious with an increase in post-resection tumour re-occurrence and the incidence of septic complications[55].

As white blood cells (WBCs) are thought to be the principle source of these alloantigens, studies have attempted to identify the effects of leucoreduction on infectious complications following cardiac surgery[55]. Although, as yet the results of this research has been inconclusive the possibility of TRIM associated post-operative infection remains a possibility and should be considered in any decision to transfuse RBCs[56;57].

1.4.2.4 Transfusion Transmitted Infection

Human immunodeficiency virus (HIV), hepatitis B, hepatitis C and human T cell lymphoma virus are all transmissible by blood. Transfusion centres in the United Kingdom screen every donation with tests for these viruses becoming ever more sensitive[58;59]. Despite this, transmission can still occur in the early phase of infection, before the antibody response has become detectable, where blood has been donated in the so called 'window period'[60].

The majority of data collected on transfusion transmitted viral infections has relied upon reported cases. This is unreliable in that many cases may remain asymptomatic or have contracted the disease by a route other than transfusion[61]. This issue was recently addressed by a survey of over five thousand patients receiving blood in the UK. Interestingly, no instances of transfusion related infection were reported although several patients had evidence of pre-transfusion infection. Where infection was hospital acquired other sources were identified[61;62]. Thus, it is reasonable to assume that viral transmission is exceedingly rare although theoretically possible.

1.4.2.5 Variant Creutzfeldt-Jakob Disease

The first description of vCJD in the UK occurred in 1996[63]. By 2001, slightly over one hundred cases had been reported although it is worth noting that the incidence of new cases has continued to decline for several years[64]. The presumed mechanism of transmission in the majority of cases is by the ingestion of beef from

cattle infected with bovine spongiform encephalopathy (BSE)[65]. Although the link with RBC transfusion has always been tenuous, this picture is now changing. Recently published data by the National Creutzfeldt-Jakob disease (CJD) Surveillance Unit has identified 3 cases of probable transfusion transmission of vCJD. To date, no evidence exists that the familial or sporadic strains of CJD may be transmitted in a similar manner[66]. The classic histological lesion evident is the presence of amyloid neural plaques surrounded by spongiform change (figure 1.3)[67]. Patients usually experience neuro-psychiatric symptoms progressing to ataxia and cognitive impairment over a period of 7-38 months. The long subclinical incubation period gives rise to concerns that large numbers of the population may be harbouring the disease asymptomatically[65;68].

At present, no specific screening test or treatment exists. As a result, strategies have now been implemented with the aim of reducing the risk of transmission. UK donor plasma has been excluded from fractionation while blood components are leucodepleted. However, despite these measures, risk can not be discounted and the possibility of transmission should be remembered by all those who prescribe blood[65;68;69].

1.4.2.6 Transfusion Related Acute Lung Injury

TRALI has been shown to be one of the leading causes of transfusion related morbidity and mortality both in the UK and US. This is despite the likelihood the condition is under reported[70–72]. The aetiology may be antibody mediated (immune) or initiated by neutrophil priming substances (non-immune). Due to the universal leucodepletion of RBCs the former is thought to be the most prevalent in the UK[70;73;74]. The incidence of immune TRALI has been placed at 1 in 625 of all patients transfused. The classical symptoms are those of acute hypoxia and non-cardiogenic pulmonary oedema, usually developing within 1-2h of the offending blood transfusion[72;75].

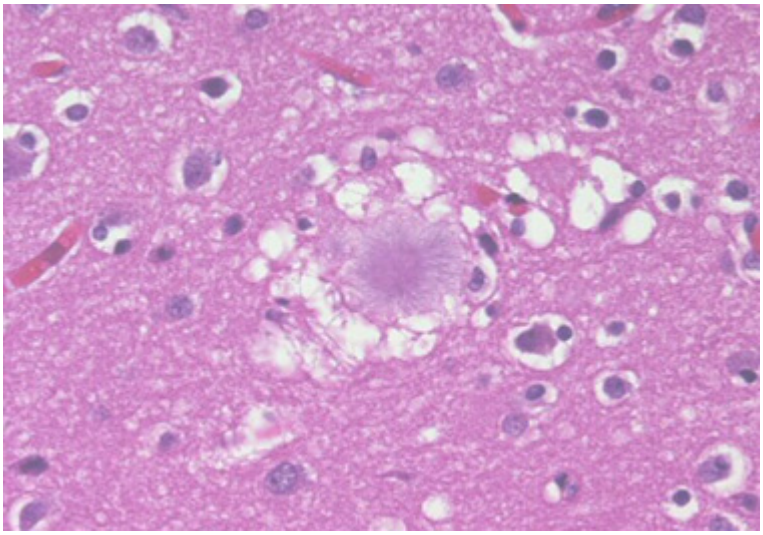


Figure (1.3) *Histological section demonstrating variant Creutzfeldt-Jakob disease (vCJD). Cerebral cortex demonstrates classic plaque structure with a glassy centre surrounded by spongiform change. Reproduced from the National vCJD Surveillance Unit, The University of Edinburgh (*`http://www.cjd.ed.ac.uk`*).*

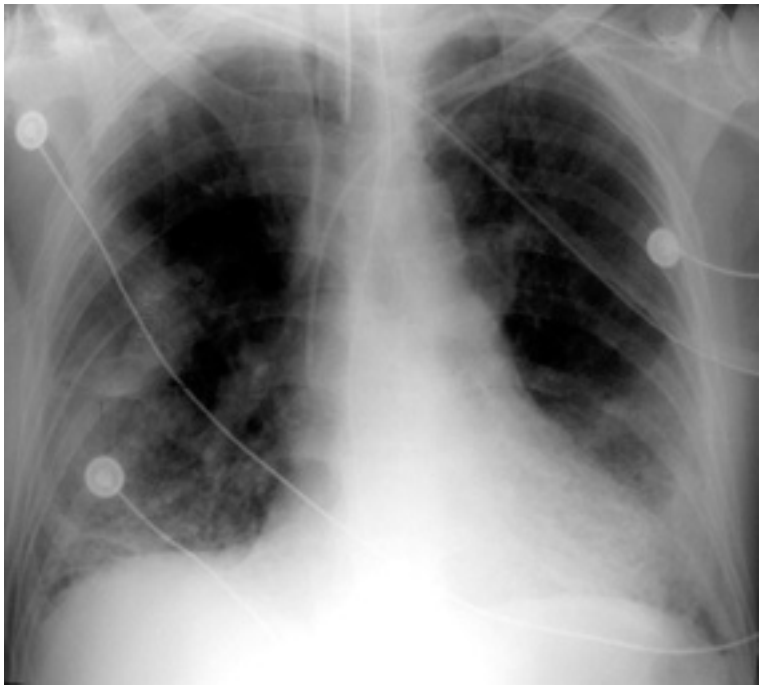


Figure (1.4) *Plain radiograph of acute lung injury (ALI). Reproduced from Medicine Net (http://www.*`medicinenet.com`*).*

Table (1.3) *Clinical diagnostic criteria for TRALI. TRALI = transfusion related acute lung injury, ALI = acute lung injury, PCWP = pulmonary capillary wedge pressure and CXR = chest x-ray.*

1. The onset of symptoms or signs is during or within 6h after the end of transfusion of one or more plasma-containing blood products
2. New onset ALI recognised by; i) Timing: Acute onset
ii) PCWP: ≤18 mmHg or no clinical evidence of left atrial hypertension
iii) CXR: bilateral fluffy infiltrates
iv) Hypoxia: Ratio of PO_2/FiO2 ≤300 mmHg regardless of positive end-expiratory pressure, or Oxygen saturation of ≤90% on room air

As defined at the Toronto consensus conference convened by the Heart, Lung and Blood Institute, April 2004.

Although the subject of much debate, at present the only diagnostic criteria for TRALI are clinical (table 1.3)[73;74;76]. Findings such as mild pyrexia, hypoxia, tachypnoea, hypotension, bilateral crepitations, bi-basal dullness and fluffy bilateral infiltrates on plain chest x-ray (CXR) may equally be attributed to CPB related acute lung injury (ALI) (figure 1.4)[77]. Furthermore, subtler forms of the condition, not complying with the above diagnostic criteria, are almost certainly overlooked. At the present time, management of TRALI is for that of ALI with ventilatory support as required. The majority of patients recover within 96h with little in the way of permanent sequelae[71].

1.4.2.7 Transfusion Associated Circulatory Overload

TACO is a term coined to describe cardiogenic pulmonary oedema following the transfusion of RBCs[78;79]. Although TACO is often confused with TRALI, the causality of the pulmonary oedema may be used to distinguish between the two conditions. The aetiology of TACO is hydrostatic while TRALI results from pulmonary endothelial damage and the resultant increase in permeability. As a result, an increase in atrial natriuretic peptide, attributable to atrial distension,

can help distinguish between the two conditions[80;81].

Although the exact incidence of TACO is unknown, it is likely an under recognised condition. Estimates vary from 1 - 8% in elderly orthopaedic patients. Invariably onset is precipitated by the rapid infusion of RBCs, often by as little as 1unit[79;82]. Clinical characteristics are those of congestive cardiac failure with hypoxia, tachypnoea and tachycardia usually evident within 1-2h of RBC transfusion. The one differentiating feature that may be noted is an increase in blood pressure. Treatment is again supportive with mechanical ventilation where required and the administration of a diuretic preparation if deemed necessary. In extreme cases venesection may be indicated[79;82].

1.4.3 Red Cell Transfusion and Survival

Although the recognised complications of RBC transfusion are rare, what about the longer term effects on patient survival? Two large retrospective analysis have associated RBC transfusion with all cause mortality following cardiac surgery. Engoren et al conducted an analysis of 1915 patients. They found that RBC transfusion was an independent mortality risk factor associated with a risk ratio of 1.7 at 5y[83]. Kuduvalli et al performed a larger analysis of 3024 patients. It was demonstrated that the mortality risk ratio, again adjusted for co-morbidity, was 1.88 at 1y[84]. In the short term, RBC transfusion has also been identified as an independent risk factor for in hospital mortality[85]. Further studies have examined the association between RBC transfusion and mortality based outcome in patients with with acute coronary syndromes (ACSs).

Wu et al reported findings in a retrospective cohort of 78,974 ACS patients. They documented a reduction in 30d mortality if patients transfused had an admission haematocrit (Hct) $< 30\%$ while no benefit was found with a Hct $> 33\%$[86]. However, two key criticisms can be leveled at this study. Firstly, patients with do not resuscitate (DNR) orders were included in the analysis. This may have influenced a transfusion practitioners decision to administer RBCs. Secondly, patients in the lower Hct groups underwent fewer coronary interventions, almost certainly influencing baseline mortality.

Perhaps a more rigorous analysis was performed by Rao et al in a smaller retrospective cohort of 24,112 patients. They found that hazard adjusted mortality in ACS patients was in fact higher when the Hct was greater than 25%. Indeed, at 30d those receiving a transfusion had a fourfold increase in mortality. This contradicts the assumption that transfusion should normalise risk in anaemic patients[87]. In the absence of large prospective analysis it is difficult to draw definitive conclusions from the studies above. However, both studies suggest that an element of caution should be applied to any decision to transfuse in this sub group of patients.

1.5 What Factors Should we Consider in Deciding to Transfuse?

1.5.1 Oxygen Transport

The role of [Hb] in transporting oxygen was recognised as early as the 1920's. Essentially, each [Hb] molecule consists of two alpha and two beta subunits, each containing a central iron based haem ring responsible for binding oxygen (figure 1.5). The vast majority of oxygen is transported in this manner, with only about 2% carried in solution under normal atmospheric conditions[88;89].

The relationship by which [Hb] binds oxygen can be represented graphically by the oxy-haemoglobin dissociation curve (figure 1.6). The curve represents the percentage saturation of [Hb] at various partial pressures of oxygen. The sigmoidal nature relates to the property of co-operative binding whereby the affinity of [Hb] for oxygen increases with each subunit that has bound an oxygen molecule. At high partial pressures, [Hb] is maximally saturated while at low partial pressures [Hb] looses its affinity for oxygen. The P50 value is often used to describe the curve and refers to the oxygen partial pressure at which [Hb] is 50% saturated. The ability of this curve to move under certain conditions is vital in the body's response to anaemia. Factors that shift the curve to the right include an increase in temperature, hydrogen iron concentration, carbon dioxide and organic phosphates, principally 2,3-diphosphoglycerate (2,3-DPG)[88]. More detail on oxygen transport is given in appendix B (page - 182).

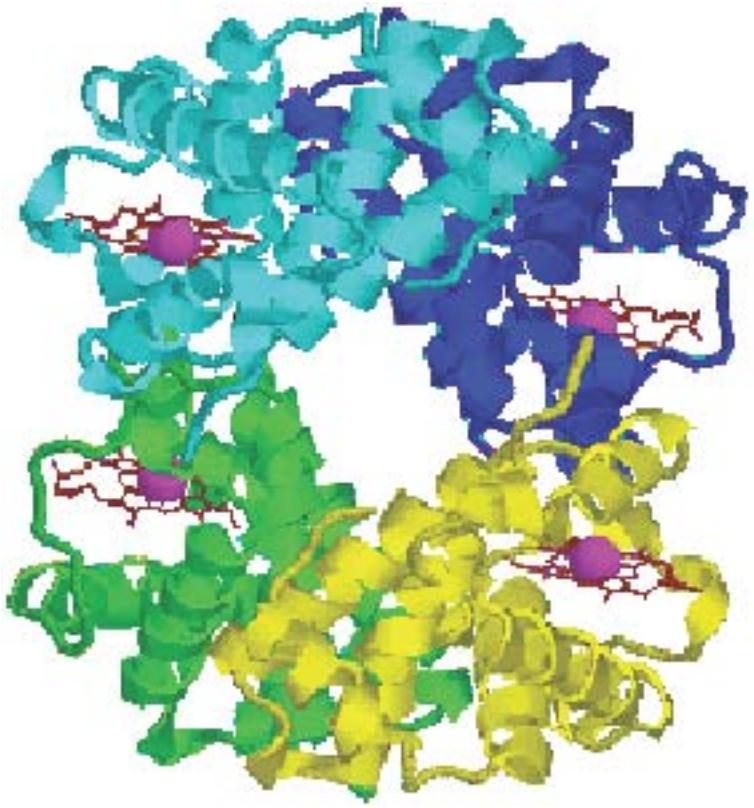


Figure (1.5) *Graphic representation of a haemoglobin molecule. Reproduced from The Biopolymer Group, Sheffield (`http://www.biopolymer.group.shef.ac.uk`).*

1.5.1.1 Supply Dependency

Systemic oxygen consumption (VO_2) is regarded as an estimate of aerobic metabolic activity within the human body. When systemic oxygen delivery (DO_2) is at normal, or supra-normal, levels, VO_2 remains constant. However, when DO_2 falls below a critical point, VO_2 may be supply limited (figure 1.7). This phenomenon has been described as pathological supply dependency as tissue oxygen extraction is unable to increase in proportion to the fall in DO_2. In such a scenario, anaerobic metabolism ensues with the accumulation of lactic acid[89;90]. Although such a model is appealingly logical, it is increasingly being questioned.

As many of the measured variables that determine VO_2 are also used in the calculation of DO_2, mathematical coupling may exist. For example, as arterial

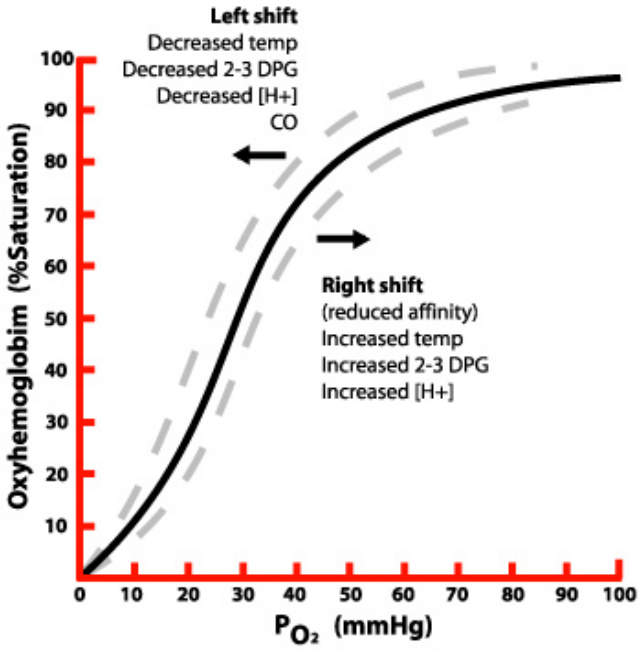


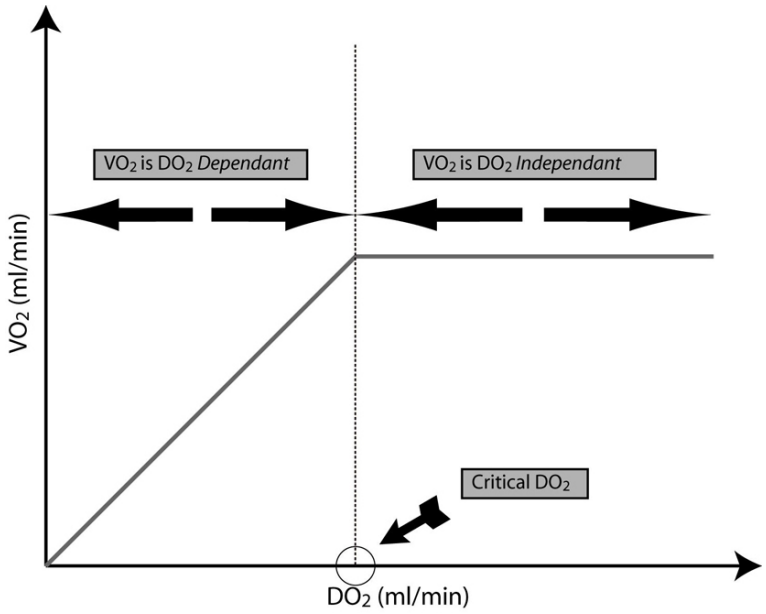
Figure (1.6) *Oxygen dissociation curve. Reproduced from Anaesthesia UK (http://www.frca.co.uk.).*

oxygen saturation (SaO_2) is found in the calculation of both VO_2 and DO_2 a mathematical link is present. This may indicate a correlation between the two variables in the absence of a true physiological association. It is now thought that this is the most likely explanation for their associated fall below a theoretical critical point[91;92]. This hypothesis is strongly supported by the observation by Myles et al that VO_2 following CPB, when measured by expired gas analysis, has no association with the value calculated by use of the reverse Fick principle[93].

1.5.1.2 Critical Oxygen Delivery

Several attempts have been made to identify when oxygen delivery becomes critical (critical oxygen delivery (DO_{2Crit})). Below this critical point metabolism can be considered to be predominantly anaerobic. Nelson et al used animal models to plot VO_2 against oxygen extraction (QO_2). The DO_2 below which VO_2 fell with

Figure (1.7) *Diagrammatic representation of pathological oxygen supply dependency.*



no further increase in QO_2 (critical oxygen extraction (QO_{2Crit})) was defined in this study as DO_{2Crit}. In the absence of sepsis, DO_{2Crit} was measured at 6.8 and 7.4ml/min/kg[94;95]. Similar findings have been reported in human models.

Shibutani et al identified a DO_{2Crit} of 330ml/min/m^2 (8.2ml/min/kg) in 58 cardiac surgery patients prior to CPB[96]. In a similar cohort, but following CPB, Komatsu et al found a value of 300ml/min/m^2 based upon measurements in 66 patients[25]. Perhaps the most rigorous study was performed by Ronco et al on a cohort of 9 non-septic, critically ill, ICU patients. VO_2, as measured by indirect calorimetry, was plotted against DO_2, as calculated by the Fick method, QO_2 and measurements of plasma lactate concentration. DO_{2Crit} was found to occur at a value of 4.5ml/min/kg[97]. All of the above studies were performed on anaesthetised patients at normothermia. In order for these values to have relevance in cardiac surgery, consideration has to be given to the marked effects of temperature in reducing VO_2 and DO_{2Crit}[89;98].

DO_{2Crit} can be plotted against actual values of DO_2 to demonstrate the presence, or otherwise, of an oxygen reserve. Such a study was performed by

Ganushchak et al with a cohort of 15 peri-operative cardiac surgery patients. The value for DO_{2Crit} was taken as that suggested by Komatsu et al, at the upper end the figures quoted. They found actual DO_2 approached DO_{2Crit} at two points: removal of the aortic cross clamp and extubation. However, at no point was systemic oxygenation impaired[89]. As it is likely that DO_{2Crit} is lower than the value used in this study, credence can be given to the theory that a significant oxygen reserve does exist in peri-operative cardiac surgery patients[97].

1.5.1.3 Lactic Acidosis

Most patients undergoing cardiac surgery will develop post-operative metabolic acidosis due to accumulation of lactate. This has been attributed to systemic hypo-perfusion and tissue hypoxia[99;100]. As cellular metabolism converts pyruvate to lactate under conditions of anaerobic metabolism it has been assumed that the magnitude of this metabolic acidosis represents the scale of the oxygen debt accrued during CPB[100]. Newer evidence however, has demonstrated that post-operative metabolic acidosis can occur under conditions of aerobic metabolism[89;101;102]. Commonly used drugs such as insulin and those containing calcium and magnesium can all lead to the deactivation of pyruvate dehydrogenase (PDH) with a resultant accumulation of lactate. Other factors include washout from previously hypo-perfused tissues and reduced lactate clearance[89;101].

It has recently been proposed that lactic acidosis following CPB may be iatrogenic[99;103]. Patients undergoing cardiac surgery receive a large amount of exogenous fluid, particularly from the pump prime during CPB. The net effect of this non-bicarbonate ion ([HCO3$^-$]) containing fluid may be to dilute extra-cellular [HCO3$^-$], thus reducing the patients own buffering capacity[104].

Traditionally, acid-base balance has been interpreted using the Henderson-Hasselbach equation. However, Stewart has proposed an alternative approach that uses fundamental laws of physical chemistry, including that of electro-neutrality, to determine exactly what variables influence hydrogen ion concentration in complex solutions. The principle components of Stewart's hypothesis are the strong ion difference , arterial partial pressure of carbon dioxide ($PaCO_2$) and the total

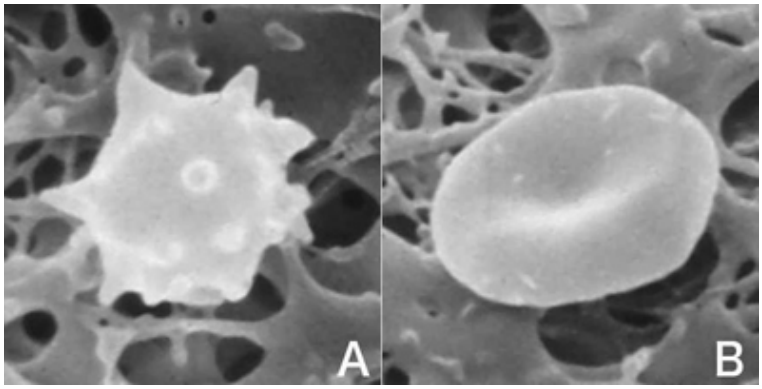


Figure (1.8) *Red cell storage lesion as viewed by electron microscopy. Image A demonstrates the typical spiculated appearance as compared to a normal red cell in image B. Reproduced by permission of Dr Katsuji Nishi, Shigi University, Japan.*

concentration of weak acids[105]. It has been demonstrated that the administration of fluids that have little or no strong ion difference, such as normal saline, reduce the strong ion difference of the blood with the net effect of an increase in the dissociation of hydrogen ions and a resultant metabolic acidosis[106–108]. Thus, metabolic acidosis after heart surgery may often reflect changes induced by administration of intra-vascular fluids that are of a low strong ion difference rather than anaerobic metabolism and accumulation of lactic acid.

1.5.1.4 Red Cell Storage

RBC transfusion is administered on the premise that it will improve tissue oxygenation. However, the evidence to support this statement is limited. Much debate exists in the literature as to the efficacy of stored RBCs in terms of oxygen transportation. Red cell 2,3-DPG is critically depleted after 10 days, which results in an increase in the affinity of the haemoglobin moiety for oxygen (figure 1.6). Morphological changes have also been noted that reduce RBC deformability and question their ability to traverse the micro-capillary bed (figure 1.8)[109;110].

There is a dearth of solid clinical data as to the effects of transfused RBCs on oxygen transport. Results from animal studies are not necessarily applicable to the human population while the methodology of the majority of human studies can be questioned due to the mathematical errors introduced when calculating

DO_2 and VO_2[91]. However, a recent RCT conducted by Walsh et al randomised 22 non-bleeding anaemic ([Hb] $<$ 9g/dl) ICU patients to receive either fresh ($\leq$ 5d) or stored ($\geq$ 20d) RBCs. No difference was found in indices of gastric and systemic oxygenation between the two groups. Also of interest was that no improvement was observed in global indices of tissue oxygenation by either group[111]. Although the [Hb] levels in this study could not be considered 'critical', it does raise the question as to the efficacy of RBCs for the transfusion threshold employed ([Hb] $<$ 9g/dl).

1.5.2 What Effect Does Lowering Haemoglobin Have on the Heart?

In healthy adults and animal models, extreme haemodilution is well tolerated by the heart. Compensatory mechanisms include; a viscosity mediated reduction in systemic vascular resistance, marked tachycardia (in non-anaesthetised individuals), an increase in stroke volume and coronary vasodilation[112]. These mechanisms appear adequate in protecting healthy hearts at a [Hb] of 5g/dl. Below this [Hb] oxygenation is impaired with sub-endocardial ischaemia and a resultant reduction in myocardial function. In addition, it has also been suggested that any electro-cardiogram (ECG) changes that do occur above a [Hb] of 5g/dl in healthy individuals may be benign and entirely reversible[113].

The above straightforward scenario, however, does not apply to individuals presenting for cardiac surgery with a variety of cardiac pathologies that may limit the patho-physiological response.

1.5.2.1 Coronary Artery Disease

Most patients presenting to a cardiac surgical unit will have coronary artery disease (CAD). Opinion as to the significance of normovolaemic anaemia with co-morbid CAD is divided although generally it has been documented that a safe transfusion threshold lies between 9-11g/dl[114].

Licker et al reported results from 44 patients scheduled to undergo CABG surgery. Individuals were randomised to receive either routine management (n =

22) or to undergo pre-operative blood donation and normovolaemic haemodilution to a Hct of 28% ([Hb] approximately 9g/dl) (n = 22). Although the echocardiographic characteristics of the intervention group changed with an increase in CO and pre-load through increased venous return, both myocardial systolic and diastolic function was comparable between the two groups. Although this study was of limited power for the outcome variables examined, it was of note no member of the intervention cohort developed evidence of myocardial ischaemia (> 1mm ECG ST segment change or left ventricular wall motion abnormality). This was despite a significant reduction in systemic DO_2 [115]. The same group, using an identical technique, have also suggested that normovolaemic hemodilution pre-CPB has a protective effect against myocardial injury [116].

Previously considered a contraindication to isovolaemic haemodilution, results have also been reported in patients with left main stem (LMS) stenosis. Herregods et al examined 71 patients with significant LMS disease (> 60% stenosis) immediately prior to CABG. When compared to controls (n = 32), patients undergoing donation and limited pre-operative haemodilution (n = 39) to a Hct of 34% ([Hb] approximately 11g/dl) exhibited no difference in significant (> 1mm) ECG ST segment change [117;118]. As hemodilution was 'limited' in this instance to provide only a small fall in Hct (6%) only limited conclusions can be drawn.

Several researchers have examined the impact of CAD on outcome following non-cardiac surgical procedures. Carson et al retrospectively studied 1958 patients undergoing major surgical procedures who refused RBC transfusion as a matter of religious choice. Those with cardiovascular disease and a low pre-operative [Hb] were found to have a greater overall mortality [119]. A limitation of this study is that only pre-operative [Hb] could be accurately considered due to incomplete data. Nelson et al conducted a small prospective observational study of 27 patients undergoing vascular surgery. They found that cardiac morbidity increased when post-operative Hct fell below 28% ([Hb] approximately 9g/dl) [120]. As the study methodology did not provide detailed documentation of the presence or absence of CAD the significance of the observations is uncertain.

The above findings may have some relevance to the general ICU setting but it is likely they are of limited applicability to cardiac surgery. Patients scheduled for valve surgery alone are likely to have had the presence of angiographically

significant CAD excluded prior to operation. Those due to undergo CABG will in the majority of cases have their coronary vascular reserve normalised [121].

1.5.2.2 Valvular Heart Disease

Experience with hemodilution and other cardiac pathologies is less well documented. Licker et al randomised 28 patients with severe aortic stenosis, scheduled for aortic valve replacement (AVR), to undergo either moderate normovolaemic haemodilution or routine management. In the study group, Hct was lowered to 28% ([Hb] approximately 9g/dl) compared with 39% ([Hb] approximately 12.9g/dl)in the control group. No difference was noted in terms of arterial blood lactate, ECG change or left ventricular wall motion abnormality. Haemodynamic compensation occurred in the study group through a viscosity-mediated reduction in systemic vascular resistance with a subsequent increase in pre-load, stroke volume and trans-valvular flow rates. It was felt however, that this response may be limited due to less efficient left ventricular stroke work [122].

Spahn et al similarly observed the effects of limited haemodilution ([Hb] = 10.3g/dl) on 20 patients with mitral insufficiency scheduled for operation. The same physiological response was observed with an increase in cardiac index (CI) due to after-load reduction and pre-load increase. Patients with chronic atrial fibrillation demonstrated a similar reserve to those in sinus rhythm [123].

1.5.2.3 Poor Ventricular Function

The reduction in viscosity associated with normovolaemic anemia results in a reduction in after-load with a resultant increase in stroke volume as outlined above. Where myocardial contractility is poor the compensatory increase in stroke volume is likely to be limited. Casutt et al studied several parameters influencing post-operative oxygen kinetics in 67 patients following cardiac surgery and found that pre-operative ejection fraction (25-87%) was not associated with post-operative DO_2 [124]. Spahn et al reported similar findings in a cohort of 90 patients. It was concluded that the compensatory mechanisms associated with hemodilution were independent of pre-operative ejection fraction over the range of 26-83% [125]. It has been commented however, that such results should be treated

with caution as few patients were found at the lower end of the ejection fraction range[126].

1.5.3 Should Haemoglobin Concentration be the Only Parameter we Consider?

It may appear to be stating the obvious that as [Hb] is a concentration it is determined by two variables, red cell volume (RCV) and plasma volume (PV). Therefore, the relative contribution of RCV and PV to the post-operative anaemia encountered in cardiac surgery patients will dictate the decision to transfuse if [Hb] is the sole parameter considered. It is often assumed that any fall in [Hb] is directly associated with RCV loss, however this is not necessarily the case. Transfusing based upon RCV loss, as opposed reduction in [Hb], will reduce the incidence of potentially unnecessary transfusion if PV expansion is a significant factor. Further improvements in such a strategy may be made if we can predict transfusion requirement based upon an individuals risk of a low post-operative RCV as opposed to a reduction in [Hb]. A final consideration is whether it is really necessary to transfuse RBCs in the absence of signs that would otherwise suggest an inadequate circulation?

1.5.3.1 The Contribution of Haemodilution to Post-operative Anaemia

Cardiac surgery, involving CPB, is associated with a heavy fluid load, both in relation to the pump priming solution and the additional intra-venous fluid administered[127;128]. If [Hb] is artificially lowered by an excessive fluid load, patients may be transfused RBCs even though RCV is only modestly reduced. In order to examine this issue, Fransen et al analysed a cohort of 20 peri-operative cardiac surgery patients. They identified a rise in PV and blood volume (BV) to a maximum of 202% and 159% respectively of the pre-operative values, 12h after removal of the aortic cross clamp (ACC). In contrast, RCV reached a low point of 95% of the pre-operative value 4h after removal of the ACC, while by 24h it had risen to 107%. Although such results need cautious interpretation as the method of calculation of RCV loss is prone to error, they suggest that PV expansion may be an important factor in influencing [Hb] and the decision to transfuse[129].

Only one study has examined peri-operative RCV change directly. In a cohort of 30 gynaecological patients, Orth et al used sodium fluorescein (NaF) and flow cytometry to conclude that Hct was a poor indicator of change in RCV[130].

1.5.3.2 Predicting Transfusion Requirement

Attempts have been made to construct predictive models for RBC requirement in cardiac surgery[131–141]. The principle aim behind these models has been the identification of high transfusion risk patients for whom pre and peri-operative strategies can be devised to minimise RBC exposure. Significant factors identified include; age, female gender, body surface area (BSA), low pre-operative [Hb], non-elective surgery, re-operation, prolonged CPB time and deep hypothermia[134;137]. In all the studies cited, the decision to transfuse was based upon measured [Hb]. Such an act may be misleading as [Hb] alone may not accurately represent peri-operative blood balance. Thus, the validity of such studies remains in question.

1.5.3.3 Indicators of an Inadequate Circulation

RBCs are often administered without due consideration to the circulatory status of the patient. In the absence of hypovolaemia, several compensatory mechanisms exist to maintain tissue oxygenation in anaemic patients (section 1.5.2). Therefore, it would seem reasonable to suggest that RBCs should only be administered when these mechanisms can no longer maintain tissue oxygenation. Madjdpour et al have suggested several parameters that may be indicate when this critical point is reached, and that can therefore be used as a guide to transfusion requirement[142;143].

These parameters are: relative tachycardia, relative hypotension, a decrease in VO_2 greater than 10% from baseline, an QO_2 greater then 50%, a mixed venous oxygen saturation (SvO_2) less than 50% and a mixed venous oxygen partial pressure (PVO_2) less than 32mmHg[142–144]. Of the parameters listed, SvO_2 may be the most valuable as it is a single variable that accurately reflects the adequacy of systemic perfusion[145]. Furthermore, as jugular venous oxygen saturation ($SJvO_2$) is consistently 5% greater than SvO_2, due to the admixture of de-oxygenated

coronary sinus blood, estimates of SvO_2 may be obtained in the absence of a pulmonary artery catheter[146;147].

In chapter 2 (page - 34), the principle points of the above discussion are incorporated into a programme of research aimed at reducing the incidence of potentially unnecessary RBC transfusion in cardiac surgery.

Chapter 2

Programme of Research

2.1 Developing a Red Cell Volume Based Transfusion Guideline

As discussed in section 1.5.3.1 (page - 31), the large fluid load associated with cardiac surgery may, mainly by lowering [Hb] through haemodilution, play a significant part in the decision to transfuse RBCs[127;128]. It is therefore the case that RCV should be given greater attention in selecting thresholds or 'triggers' to guide the decision to transfuse RBCs[112;113]. Developing and incorporating such a strategy into everyday clinical practice is no easy task as little or no data exists in the current literature.

In section 1.5.3.2 (page - 32), we mentioned considering patient and operative variables that may be associated with RBC transfusion where a [Hb] threshold is the only trigger for transfusing. Such associations have been explored in the past with the aim of tailoring peri-operative management to minimise exposure to allogenic RBCs[134;137]. In chapter 3 (page - 40), consideration is given to the role of parameters that may influence both RCV and PV, and hence RBC transfusion, through a large retrospective analysis.

This idea is developed in the subsequent two chapters. In chapter 4 (page - 53), we examine variables associated with loss of RCV, and therefore more likely to be representative of peri-operative RCV balance, in a simple observational study. The second issue we consider is the relative contribution of haemodilution to post-operative anaemia. If, as may be expected, RCV is relatively well preserved in the presence of PV expansion, then allowing for this haemodilution before administering RBCs will allow a more evidence based approach to transfusion[129]. In chapter 5 (page - 66), we examine this hypothesis using the data set from the study cohort employed in chapter 4 (page - 53).

Traditionally, measurement of RCV has involved the use of chromium labelled RBCs. Unfortunately this approach can not be applied to the rapidly changing peri-operative situation as it is not possible to make repeated measurements over short time periods due to persistence of the isotopic label. Recently however, Lauermann et al reported results with the use of NaF flow cytometry as an alternative technique for RCV estimation. The principle advantage is that this technique may be repeated after 1h and as such may be applied in the surgical

setting[130;148]. As the methodology used in chapters 4 (page - 53) and 5 (page - 66) relies upon the use of swab weight/drain volume and its measured Hct for calculating RCV loss it may be prone to a degree of measurement error. In chapter 6 (page - 77) we attempt to validate the technique proposed by Lauermann et al in order to allow for a more accurate calculation of peri-operative RCV loss.

There are two further important questions if RCV is to be considered in the transfusion decision making process. First, if it can be demonstrated that haemodilution is a significant factor in the peri-operative depression of [Hb], then as normal circulatory physiology is restored, does [Hb] recover[127;128]? The second is to ensure that if lower [Hb]s are to be tolerated peri-operatively, patients are not compromised in terms of critical requirement for oxygen[89;97]. Both these questions are addressed in chapters 7 (page - 83) and 8 (page - 94) respectively.

2.2 Lung Injury Following Red Cell Transfusion

ALI is a common occurrence following cardiac surgery. Causative factors may include; pre-operative airways disease, smoking, the CPB circuit, sepsis, RBC transfusion and ventilatory barotrauma. In its minor manifestations patients may experience mild breathlessness. Those less fortunate, approximately 2%, may develop the more acute form leading to prolonged ventilation and in 50% of cases death[77;149]. Lung injury occurs due to the complex interaction of pro-inflammatory mediators including; cytokines, complement, neutrophils, monocytes, activated endothelial cells and platelets. Neutrophils deposited in the lung cause widespread parenchymal injury through the release of elastase and oxygen free radicals[150].

As mentioned in section 1.4.2.6 (page - 18), when considering ALI in relation to RBC transfusion the term TRALI is usually applied. However, TRALI only describes the acute form of the condition. Subtler forms of ALI may well occur though they have not been described in relation to RBC transfusion[74]. This almost certainly relates to the difficulty in making a sub-clinical diagnosis of ALI. In the past, various inflammatory mediators have been measured but these may

not be specific for the degree of pulmonary endothelial damage[151]. In attempting to address this issue in relation to CPB mediated ALI several authors have explored the role of exhaled nitric oxide (NO) analysis[152–154].

The rationale behind this approach is that any reduction in peri-operative exhaled NO is likely to be a direct reflection of the degree of pulmonary endothelial damage as the source of NO production is lost[152–154]. Although NO is produced by several other sources in the lung, such as the bronchial epithelium, it's diffusibility ensures that a reduction from any one source is detectable clinically[153]. It should be remembered that reduction in NO within the pulmonary vasculature is thought to be the causative mechanism for an increase in pulmonary vascular resistance (PVR) in cases of ALI. This provides the basis for the therapeutic administration of inhaled NO to these individuals[155].

In chapter 10 (page - 122), we conduct a small pilot study aimed at exploring the association between transfused RBCs and exhaled NO.

2.3 Improving Anti-coagulation Strategies for Cardio-pulmonary Bypass

Patients undergoing cardiac surgery, with the use of CPB, will consume coagulation factors to a varying extent. This is a potentially detrimental scenario which, in its extreme form, may result in disseminated intravascular coagulation (DIC) and complications such as bleeding, microvascular thrombosis and renal failure[156]. To minimise this risk, numerous anti-coagulation monitoring strategies have been devised to control heparin administration during cardiac surgery[157–159]. At present, activated clotting time (ACT) is the most frequently used method of assessing the adequacy of heparinisation. Advantages include its; simplicity, cost and relative effectiveness[158;160;161]. However, it may give misleadingly high prolongations of clotting time during conditions of haemodilution and hypothermia i.e. as encountered during CPB[162–164]. The resultant possibly inadequate heparinisation, with exposure of blood to foreign surfaces, may lead to excessive coagulation activation and clotting factor loss[165]. Some investigators have thus

advocated the use of individualised heparin management systems (HMSs) (figure 11.1, page - 136)[164;166–169].

HMS provides in vitro analysis of individual heparin dose responsiveness (HDR) allowing patient specific administration of loading dose heparin prior to CPB. Blood heparin concentration (BH_{Conc}) is monitored during CPB by a heparin protamine titration (HPT) technique allowing early detection and correction of sub-therapeutic anti-coagulation. Following the discontinuation of CPB, HPT is again used to provide an accurate protamine dose calculation. Residual circulating heparin following protamine administration can also be detected. Proposed benefits include a reduction in coagulation activation, blood loss and post-operative blood product requirement[164;166–169]. Despite this, HMSs have not been widely used to replace ACT as the standard heparin monitoring and dosage system. Reasons may include cost and the cumbersome testing process involved. Previous research has demonstrated that the key difference between ACT and HMS based anti-coagulation is that the latter receive a greater dose of heparin, primarily during CPB[166–168]. Thus it appears reasonable to hypothesise that the administration of supplemental heparin, independent of ACT, may confer similar benefits to HMSs.

In chapter 11 (page - 132), we examine the relative merits of a modified ACT based anti-coagulation system for CPB as compared to a HMS.

Part II

Methods and Results

Chapter 3

Patient and Procedural Variables Associated with Red Cell Transfusion: A Retrospective Analysis

3.1 Background

The first step in any programme of research is to understand the basis for the current issue. In this case that equates to answering the question of why cardiac surgery is a heavy user of RBCs[4]. A good basis on which to begin to attempt to answer this question is the observation that most centres consider [Hb] alone when deciding to administer RBC transfusions. Presumably, this is based upon the assumption that [Hb] is an accurate indicator of peri-operative RCV balance[22;23;25]. As discussed in section 1.5.3 (page 31), there is currently no justification for this assumption in the peer reviewed literature. In order to accurately determine the role of [Hb] in RBC transfusion we need first to consider that laboratory assays measure [Hb] in a sample of patients whole blood. [Hb] may be affected by both RCV and PV. When, as is usual, [Hb] is taken as the threshold or 'trigger' for administering RBCs, it is the relative balance between these two parameters that determines when an RBC transfusion is given[129;130]. Theoretically, a patient may receive an RBC transfusion in response to a heavy fluid load, despite the loss of little RCV. This scenario occurs in cardiac surgery in relation to the use of CPB[127;139].

This study was designed to establish what patient and procedural variables are associated with RBC transfusion through a large retrospective analysis with the intention of gaining a greater insight into the relative contribution of RCV and PV to post-operative anaemia.

3.2 Aim

To retrospectively analyse what patient and operative variables are associated with RBC transfusion.

3.3 Materials and Methods

3.3.1 Patients

As this was a retrospective analysis ethical approval was not required. Data from all adult (age $\geq$ 16y) cardiac surgery patients presenting for operation between January 2003 and December 2006 was included in the analysis. No exclusion criteria were used. In total, 2399 data sets were collated.

3.3.2 Study Variables

For the purposes of the analysis, the variables outlined below were collected to form each patient data set (tables 3.1 and 3.2). Data was collected from internally held audit records, the Scottish Audit of Surgical Mortality (SASM) and the SNBTS collated better blood database. The variables collected were divided into continuous and categorical parameters. A further division was then made into; patient factors, pre-operative variables and procedural variables. All definitions given in the tables of variables examined were obtained from the Scottish Cardiac Audit Definitions Database. Where body surface area is mentioned, this was calculated as recommended by Gehan and George[170]. RCV was determined by the formulae recommended by the International Committee on Standardisation in Haematology (ICSH) (equations A.2 and A.3)[171].

3.3.3 Red Cell Transfusion

The transfusion of RBCs was guided by local departmental protocol (table 4.1).

3.3.4 Statistical Analysis

The primary dependant variable for all analysis was whether or not patients were transfused RBCs. All continuous variables were first analysed by Q-Q plot. This test compares the quantile's (Q) of the sample data against those of the standard normal distribution. Where normal distribution was evident the two group Students t-test was used, provided the further assumptions were met i.e. homogeneity of variance. Where the data was non normal, or the assumptions of the t-test were not met, Mann-Whitney U test was used. Normally distributed data was presented as mean (standard deviation (SD)) with non normal data as median (standard error of the mean (SEM)). For the same dependant variable, categorical data was examined by chi-squared test and expressed as number (%). In addition to the above, Spearman rank correlation coefficient (r) were calculated for the continuous variables with the number of units RBCs transfused. A P value (P_{Val}) ≤ 0.05 was considered statistically significant. SPSS version 13 was used for all statistical analysis.

3.4 Results

Of the retrospective data sets collected 1363 (56.8%) of patients were transfused RBCs versus 1036 (43.2%) who were not. On average, individuals received a mean of 2.5u per patient operated while those that received a transfusion were given a mean of 4.3u.

3.4.1 Study Variables vs. Red Cell Transfusion Status

Analysis of all continuous variables versus RBC transfusion status is presented in table 3.3. Of those parameters examined only; weight, BSA, creatinine and Euro score met the assumptions of the two group t-test. The remainder were examined by Mann-Whitney U test.

Table (3.1) *Demographics and pre-operative study variables. NYHA = New York Heart Association, MI = myocardial infarct and IABP = intra-aortic balloon pump.*

Variable	**Definition/Unit of Measurement**	**Class**
Patient Variables		
Age	Years	Continuous
Gender	Male/Female	Categorical
Height	Metres	Continuous
Weight	Kilograms	Continuous
Body Surface Area	$Metres^2$	Continuous
Red Cell Volume	Millilitres	Continuous
Pre-Op Variables		
Surgical Priority	1 = Elective, 2 = Expedite 3 = Emergency	Categorical
Euroscore	Numerical Scale	Continuous
NYHA Class	Dyspnoea/Angina on Effort; 1 = Strenuous, 2 = Moderate, 3 = Mild, 4 = Rest	Categorical
Angina Class	Pain on Effort; 1 = Strenuous, 2 = Moderate, 3 = Mild, 4 = Rest	Categorical
Haemoglobin	Grams/Decilitres	Continuous
Creatinine	μ mol/Litre	Continuous
Previous MI	Yes/No	Categorical
Poor Left Ventricle	Ejection Fraction < 30%; Yes/No	Categorical
IABP	Yes/ No	Categorical
Peripheral Vasc Dis	Yes/No	Categorical
Poor Left Ventricle	Ejection Fraction < 30%; Yes/No	
Diabetes	Yes/No	Categorical
Smoking	0 = Non, 1 = Ex (Stopped >1 Month), 2 = Current (Within 1 Month)	Categorical

Table (3.2) *Procedural study variables. CABG = coronary artery bypass grafting, AVR = aortic valve replacement, MVR = mitral valve repair/replacement, TVR = tricuspid valve repair/replacement, CPB = cardio-pulmonary bypass and ACC = aortic cross clamp*

.

Variable	**Definition/Unit of Measurement**	**Class**
Operation Type		
CABG		Categorical
Valve	AVR, MVR, TVR	Categorical
CABG + Valve	Combination of CABG + AVR/MVR/TVR	Categorical
Double Valve	Any Combination of AVR/MVR/TVR	Categorical
Procedural Variables		
Redo	> First Time Operation; Yes/No	Categorical
Hardy Class	1 = 1st CABG, 2 = 1st Valve, 3 = Redo CABG, 4 = Redo Valve or Combined	Categorical
CPB Time	Minutes	Continuous
ACC Time	Minutes	Continuous
CABG Only		
CPB Status	On/Off Pump Surgery	Categorical
Triple Vessel	Disease > 75% in 3 Vessels; Yes/No	Categorical
Left Main Stem	Disease > 50% in Left Main Stem; Yes/No	Categorical

Table (3.3) *Analysis of continuous variables. P_{Val} = P value, BSA = body surface area, RCV = red cell volume, CPB = cardio-pulmonary bypass and ACC = aortic cross clamp. As the majority of the variables were non-normally distributed data is presented as median (SEM) unless otherwise indicated.*

Parameter	**Transfused (n =1363, 56.8%)**	**Not Transfused (n = 1036, 43.2%)**	**P_{Val}**	**Missing (n)**
Patient Variables				
Age (y)	69.0 (0.26)	65.0 (0.33)	<0.001	2
Height (m)	1.68 (0.003)	1.70 (0.003)	<0.001	131
Weight (kg)*	76.8 (14.8)	83.2 (15.2)	<0.001	95
BSA (m^2)*	1.86 (0.21)	1.94 (0.20)	<0.001	138
RCV (ml)	1885 (10.0)	2035 (10.9)	<0.001	138
Pre-Op Variables				
Creatinine (μmol/L) *	107 (44)	105 (55)	0.41	133
Haemoglobin (g/dl)	13.4 (0.05)	14.3 (0.05)	<0.001	169
Euro Score*	4.6 (2.9)	3.5 (3.0)	<0.001	632
Procedural Variables				
CPB Time (min)	110 (1.6)	98 (1.7)	<0.001	0
ACC Time (min)	69 (1.1)	62 (1.2)	0.001	0

* As this variable was normally distributed data is presented as mean (SD).

Table (3.4) *Analysis of categorical patient variables. P_{Val} = P value, MI = myocardial infarction, PVD = peripheral vascular disease, COAD = chronic obstructive airways disease and LV = left ventricle. All data is presented as n (%).*

Parameter	**Transfused (n =1363, 56.8%)**	**Not Transfused (n = 1036, 43.2%)**	**P_{Val}**	**Missing (n)**
Gender				
Male	912 (52.6%)	822 (47.4%)	<0.001	4
Female	449 (67.9%)	212 (32.1%)		
Prev MI				
Yes	485 (55.0%)	397 (45.0%)	<0.001	59
No	849 (58.2%	609 (41.8%)		
PVD				
Yes	268 (59.9%)	179 (40.1%)	<0.001	2
No	1093 (56.0%)	857 (44.0%)		
COAD				
Yes	125 (60.4%)	82 (39.6%)	<0.001	2
No	1236 (56.4%)	954 (43.6%)		
Diabetes				
Yes	260 (61.9%)	160 (38.1%)	<0.001	2
No	1101 (55.7%)	876 (44.3%)		
Poor LV				
Yes	80 (67.2%)	39 (32.8%)	0.008	143
No	1195 (55.9%)	941 (44.1%)		
Smoking				
Non	455 (59.6%)	308 (40.4%)	0.09	299
Ex	720 (56.2%)	561 (43.8%)		
Current	152 (50.8%)	147 (49.2%)		

Table (3.5) *Analysis of categorical pre-operative variables. P_{Val} = P value, NYHA = New York heart association and IABP = intra-aortic balloon pump. All data is presented as n (%).*

Parameter	**Transfused (n =1363, 56.8%)**	**Not Transfused (n = 1036, 43.2%)**	**P_{Val}**	**Missing**
Surgical Priority				
Elective	935 (54.3%)	786 (45.7%)	<0.001	2
Expedite	386 (62.1%)	236 (37.9%)		
Emergency	39 (73.6%)	14 (26.4%)		
Angina Class				
Class 1	138 (53.3%)	121 (46.7%)	0.31	589
Class 2	302 (52.8%)	270 (47.2%)		
Class 3	329 (54.9%)	271 (45.1%)		
Class 4	227 (59.9%)	152 (40.1%)		
NYHA Class				
Class 1	351 (54.5%)	293 (45.5%)	0.03	30
Class 2	380 (54.0%)	323 (46.0%)		
Class 3	450 (57.7%)	330 (42.3%)		
Class 4	166 (68.6%)	76 (31.4%)		
Pre-Op IABP				
Yes	51 (69.9%)	22 (30.1%)	<0.001	1
No	1310 (56.4%)	1014 (43.6%)		

Table (3.6) *Analysis of categorical operative variables. P_{Val} = P value, CABG = coronary artery bypass grafting, AVR = aortic valve replacement and MVR = mitral valve repair/replacement. All data is presented as n (%).*

Parameter	**Transfused (n =1363, 56.8%)**	**Not Transfused (n = 1036, 43.2%)**	**P_{Val}**	**Missing (n)**
Operation				
CABG	841 (52.5%)	762 (47.5%)	<0.001	1
AVR	188 (60.8%)	121 (39.2%)		
MVR	91 (61.9%)	56 (38.1%)		
CABG + AVR	152 (71.0%)	62.0 (29%)		
CABG + MVR	48 (64.6%)	17 (35.4%)		
AVR + MVR	15 (33.3%)	10 (66.7%)		
Redo				
Yes	66 (67.3%)	32 (32.7%)	<0.001	2
No	1295 (56.3%)	1004 (43.7%)		
Hardy				
Score 1	818 (52.0%)	755 (48.0%)	<0.001	2
Score 2	250 (60.7%)	162 (39.3%)		
Score 3	22 (71.0%)	9 (29.0%)		
Score 4	271 (71.1%)	110 (28.9%)		

The principle findings were that; increasing age, small height/weight/BSA/RCV, low pre-op [Hb], high Euro score and prolonged CPB/ACC time were all identified as risk factors for receiving an RBC transfusion ($P_{Val} \leq 0.001$). Creatinine was the only non-significant continuous variable examined ($P_{Val} = 0.41$).

Analysis of all categorical variables versus RBC transfusion status is presented in tables 3.4, 3.5, 3.6 and 3.7. All parameters were examined by chi-square test. For the patient variables, the presence of; female gender, previous myocardial infarction (MI), peripheral vascular disease (PVD), chronic obstructive airways disease (COAD), diabeties and poor left ventricle were found to be significant at

Table (3.7) *Analysis of categorical operative variables for CABG only. CABG = coronary artery bypass grafting, P_{Val} = P value and CPB = cardio-pulmonary bypass . All data is presented as n (%).*

Parameter	**Transfused (n = 835, 52.3%)**	**Not Transfused (n = 769, 47.7%)**	**P_{Val}**	**Missing (n)**
3 Vessel Disease				
Yes	614 (52.2%)	562 (47.8%)	0.83	1
No	225 (52.6%)	203 (47.4%)		
Left Main Stem				
Yes	286 (51.2%)	272 (48.8%)	0.324	1
No	553 (52.9%)	493 (47.1%)		
CPB Status				
On Pump	73 (22.5%)	251 (77.5%)	<0.001	1
Off Pump	766 (59.8%)	514 (40.2%)		

the 5% level ($P_{Val} \leq 0.008$). No such association was found with smoking habit ($P_{Val} = 0.09$) (table 3.4).

Similar findings were posted for the pre-operative factors of; increasing surgical priority and the presence of an intra-aortic balloon pump (IABP) ($P_{Val} < 0.001$). New York Heart Association (NYHA) class did reach statistical significance although this was not as strong an association as the aforementioned variables ($P_{Val} = 0.03$). Angina class was found to be non-significant ($P_{Val} = 0.31$) (tables 3.1 and 3.5).

When considering the operative procedure, those of a more complex and prolonged nature were likely to result in an outcome of RBC transfusion. Maximal risk was found for combined CABG and AVR procedures with 72% transfused. This is compared to a rate of 52.5% for straightforward CABG procedures ($P_{Val} < 0.001$). The further indicators of operative complexity examined (Hardy classification and redo status) were both also highly significant predictors of RBC transfusion ($P_{Val} < 0.001$) (tables 3.1 and 3.6).

Table (3.8) *Correlation coefficient (r) for continuous variables vs RBCs (u) transfused. P_{Val} = P value, CPB = cardio-pulmonary bypass and ACC = aortic cross clamp.*

Parameter	**Correlation Coefficient (r)***	**P_{Val}**
Age (y)	0.23	<0.001
Height (m)	-0.15	<0.001
Weight (kg)	-0.24	<0.001
Body Surface Area (m^2)	-0.24	<0.001
Red Cell Volume (ml)	-0.23	<0.001
Creatinine (μmol/l)	0.06	0.04
Haemoglobin (g/dl)	-0.31	<0.001
Euro Score	0.28	<0.001
CPB Time (min)	0.21	<0.001
ACC Time (min)	0.16	<0.001

* Given the non-linearity of the data analysed Spearman's rank correlation test was used.

Of the parameters that could only be analysed for CABG procedures, CPB status (on or off pump) was the only significant variable($P_{Val} < 0.001$). The presence of LMS ($P_{Val} = 0.324$) or 3 vessel disease ($P_{Val} = 0.83$) was non-significant at the 5% level (table 3.7).

3.4.2 Continuous Variables vs. Red Cell Units Transfused

The correlation of the continuous variables with the number of units RBC transfused produced similar findings to those found with RBC transfusion status. Individuals of advancing age with small height/weight/BSA/RCV and prolonged CPB/ACC times were more likely to receive a greater number of RBCs ($P_{Val} < 0.001$). The strongest correlation was identified with pre-operative [Hb] (r = -0.31, $P_{Val} < 0.001$). Of notable difference in this analysis was that creatinine did reach statistical significance ($P_{Val} = 0.04$) (table 3.8).

3.5 Summary of Principle Findings

Off the continuous variables examined it was evident that; advancing age, small body size and low pre-operative [Hb] were all significantly associated with RBC transfusion status and the number of units RBCs transfused. This was also true of the continuous variables of CPB and ACC time that were indicative of a prolonged operative time. Creatinine was associated with units RBC transfused but not transfusion status. When considering the categorical patient parameters, it was evident that those individuals with any pre-operative morbidity were at greater risk of receiving RBCs. The exception to the rule was pre-operative smoking status. Factors that were indicative of clinical urgency for operation also carried a greater attendant risk for RBC transfusion. Similarly, this was also found where the procedural complexity increased.

Please see section 12.1 (page - 152) for a full discussion of the above findings.

Chapter 4

Factors Predicting Loss and Gain of Red Cell Volume

4.1 Background

[Hb] is currently considered the most useful investigation on which to base RBC transfusion . Several studies have developed predictive models based upon [Hb] for blood product requirement in the cardiac surgery patient[131–141]. The aim has been to tailor peri-operative management in order to minimise transfusion whilst increasing blood ordering efficiency . The core assumption made by many of these authors is that [Hb] accurately reflects peri-operative blood balance and change in RCV. However, the dilutional effects of the CPB circuit, along with the additional fluid received post-operatively, may depress [Hb] irrespective of RCV status[129;130;139]. Thus some doubt can be cast on the efficacy of such models.

This study was designed to establish what patient and procedural variables are associated with loss of RCV as opposed to a reduction in [Hb]. In addition, we also determined which of these variables are associated with RBC transfusion when a [Hb] transfusion trigger was the sole parameter considered.

4.2 Aim

To determine what pre and intra-operative variables are associated with loss or gain in RCV due to bleeding or RBC transfusion.

Table (4.1) *RBC transfusion protocol. RBC = red blood cell, Hb = haemoglobin concentration, CPB = cardio-pulmonary bypass and ICU = intensive care unit.*

Time	**Transfuse if Hb (g/dl) <***
During CPBn	**6**
< 4h Post ICU Transfer	**8**
> 4h Post ICU Transfer	**9**

* This system was adopted from Scottish Intercollegiate Guideline Network (SIGN) number 54 (`http://www.sign.ac.uk`)[172].

4.3 Materials and Methods

4.3.1 Patients

Ethical approval was gained from Lothian Regional Ethics Committee (LREC). All adult (age $\geq$ 16y) elective cardiac surgery patients presenting for operation between August and October 2003 were considered for recruitment. Exclusion criteria were; inability to provide informed consent, pre-operative anaemia ([Hb] $<$ 13g/dl for males and $<$ 11g/dl for females), emergency surgery, redo surgery and surgical re-exploration.

Figure (4.1) *Bayer Healthcare Rapidlab 855 Arterial Blood Analyser.*

4.3.2 Patient Management

Anaesthesia was induced and maintained with a combination of; fentanyl, midazolam, propofol, enflurane and pancuronium. Prior to the commencement of surgery standard monitoring was instituted (arterial pressure, central venous pressure, electrocardiogram, urinary catheter and nasal temperature). Heparin was given at a dose of 300 International Units (IU)/kg to provide anti-coagulation for CPB. An ACT in excess of 480s was maintained in all cases with supplemental heparin administered where required as per anaesthetic preference. The extracorporeal circuit consisted of a roller pump*, flexible venous reservoir, vent and cardiotomy suction†. The circuit was primed with 2l Hartmann's solution, 50mmol bicarbonate, 150mmol mannitol and 8000IU of heparin for a total volume of 2250ml. Once CPB had been established, and the ACC applied, cold blood cardioplegia was delivered to achieve cardiac arrest. When the procedure had been completed, patients were rewarmed fully to 37^0C and CPB discontinued. Protamine sulphate was given at a dosage ratio of 1:1 of the initial heparin bolus dose. Additional protamine was given where the ACT remained in excess of 140s. All available 'pump blood' remaining within the CPB circuit was returned to the patient at the end of CPB.

Thoracic drainage was achieved with a combination of pericardial, mediastinal and pleural drains where required. All drains were placed on continual suction maintained at 20cmH_2O. Plain chest radiographs were examined post-operatively to ensure the absence of a significant thoracic collection. Crystalloid was given in the ICU at a rate of 0.5ml/kg/h with colloid administered (4.5% human albumin solution) where required for hypotension (mean arterial pressure (MAP) $<$ 60mmHg) and/or poor urine output ($<$ 0.5ml/kg/h).

4.3.3 Red Cell Transfusion

All RBC transfusions were given strictly as dictated by local protocol as determined by the principal investigator (table 4.1).

*Stockert Instruments, Munich, Germany.

†Medtronic Inc, Minneapolis, USA.

Figure (4.2) *Ohaus Navigator Precision Balances.*

4.3.4 Calculation of Red Cell Volume Loss and Gain

The following peri-operative time points were identified for the collection of study data; preoperative, induction, incision, on-CPB, off-CPB, return to the intensive care unit (ICU_{Ret}) and 1,3,6,10,16 and 24h post return to the ICU. RCV loss was calculated from; swab weight, drape weight and arterial Hct at the time of collection and; thoracic effluent volume, non-returned CPB volume, blood sample volume and the directly measured Hct. Whole blood Hct was measured by an arterial blood analyser (figure 4.1)*. Effluent Hct was calculated by the centrifugation (6000rpm for 10m) of a 3ml sample† . It should be noted that effluent was only sampled following thorough mixing to ensure a homogeneous solution. All weights were recorded to 0.01gm by the use of precision balances (figure 4.2)‡.

Although RBC units are not constant in their Hct or volume, each unit of RBCs transfused was taken to represent an RCV gain of 168ml. This figure is the average RCV for units provided by the SNBTS. The RCV content of returned

*Rapidlab 855 Arterial Blood Analyser, Bayer HealthCare, Leverkusen, Germany.
†CEP 2000 Centrifuge, Capricorn Laboratory Equipment, Ringwood, UK.
‡Navigator N08110 Balances, Ohaus Corporation, Pine Brook, USA.

pump blood was calculated from its measured volume and Hct.

4.3.5 Patient and Operative Variables

Previous studies have identified several factors that may affect RCV loss/gain from induction of anaesthesia (defined for the reporting of results as t = 0) until 24h postoperatively and also from ICU_{Ret} until the same time point[131–141]. These are; age, sex, height, weight, BSA, initial patient RCV, Hct at induction and anti-platelet therapy taken within seven days prior to surgery. Additionally, operative factors that may affect loss or gain of RCV from chest closure were identified as; CPB time, ACC time, minimum temperature, max re-warm temperature, loss of RCV from induction to closure and the volume of non-blood fluid administered for the same time period. In the present study, the initial patient RCV was calculated as described by the ICSH (equations A.2 and A.3)[171]. BSA was determined as recommended by Gehan and George (equation A.1)[170].

4.3.6 Statistical Analysis

All variables were presented as mean (SD). To examine the association between the numerical variables outlined above and RCV loss, linear regression analysis was used. Pearson's rank correlation was performed for the RCV gain data. To determine the significance of any association between RCV loss/gain the binary parameters of sex and anti-platelet therapy a Wilcoxon's signed ranks test was used. A $P_{Val} \leq 0.05$ was considered statistically significant. SPSS version 13 was used for all statistical analysis.

Table (4.2) *Patient and operative characteristics. BSA = body surface area, Hct = haematocrit, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, MVR = mitral valve repair/replacement, CPB = cardio-pulmonary bypass and ACC = aortic cross clamp. All data is presented as mean (SD).*

Patient Variables (n = 29)	Age (y)	65 (9.6)
	Gender (m/f)	18/11
	BSA (m^2)	1.9 (0.3)
	Pre-operative Hct (%)	40.7 (3.4)
Operative Variables (n = 29)	CABG	23
	AVR	3
	MVR	1
	CABG + MVR	1
	AVR + MVR	1
	CPB Time (min)	91 (32)
	ACC Time (min)	53 (24)
	Min Temp (^{0}C)	31.7 (1.6)
	Max Temp (^{0}C)	37.2 (0.3)

4.4 Results

4.4.1 Demographic and Peri-operative Data

Thirty-two adult elective cardiac surgery patients were recruited to the study over a three month period. Three patients were subsequently excluded due to surgical re-exploration for bleeding. Of the twenty-nine patients with complete data, 23 underwent CABG, 3 a AVR, 1 a mitral valve repair/replacement (MVR), 1 a CABG and MVR and 1 a AVR and MVR. The mean age was 65y (SD 9.6) with a BSA of 1.9m^2 (SD 0.3) and pre-operative Hct of 40.7% (SD 3.4). Eighteen of the patients were male, eleven female. The mean CPB time was 91min (SD 32) with the ACC applied for 53min (SD 24). The minimal temperature on cooling was 31.7^{0}C (SD 1.6) with a max re-warm temperature of 37.2^{0}C (SD 0.3) (table 4.2).

Table (4.3) *Red cell volume loss and clear fluid administration until t = 24h. RCV = red cell volume and ICU = intensive care unit. All data is presented as mean (SD).*

RCV Loss (ml)	Theatre	450 (126)
	ICU	205 (113)
	Total	655 (161)
Fluid Administration Theatre (ml)	Colloid	266 (383)
	Crystalloid*	5470 (1178)
	Total	5736 (1231)
Fluid Administration t = 0 - 24h (ml)	Colloid	2111 (912)
	Crystalloid*	6699 (1350)
	Total	8810 (2262)

* This figure includes the 2250ml extra-corporeal circuit prime.

From t = 0 - ICU_{Ret} mean RCV loss was 450ml (SD 126) with a further 205ml (SD 113) lost from t = ICU_{Ret}- 24h. There was no significant difference noted in terms of RCV loss between the four surgeons taking part in the study. Clear fluid administration from t = 0 - 24h was 2111ml (SD 912) for colloid and 6699ml (SD 1350)* for crystalloid (table 4.3). Over the same time period, 79% (n = 23/29) of patients were transfused a mean of 2.4u (SD 1.1) of RBCs with a further 6.9% (n = 2/29) receiving 5u (SD 1.4) of fresh frozen plasma (FFP) and 13.3% (n = 4/29) given 1.3u (SD 0.5) of platelets (table 4.4).

4.4.2 Factors Affecting Red Cell Loss

4.4.2.1 Time = 0 - 24h

The variables of; age (r = 0.29, P_{Val} = 0.12), height (r = 0.19, P_{Val} = 0.32), weight (r = 0.08, P_{Val} = 0.68), BSA (r = 0.1, P_{Val} = 0.6), initial patient RCV (r = 0.22, P_{Val} = 0.25) and induction Hct (r = 0.27, P_{Val} = 0.16) showed no significant association with RCV loss. Similarly, no significant difference was

*This figure includes the 2250ml extra-corporeal circuit prime.

Table (4.4) *Red cell transfusion data until t = 24h. RBC = red blood cell, FFP = fresh frozen plasma, Plt = platelets. All data is presented as mean (SD).*

		Units Per Patient	
	% of Patients	**Overall**	**Transfused**
RBC	79.3	1.9 (1.4)	2.4 (1.1)
FFP	6.9	0.4 (1.3)	5 (1.4)
Plt	13.8	0.2 (0.5)	1.3 (0.5)

observed with gender (m = 697ml SD 177, f = 587ml SD 107, P_{Val} = 0.24) nor those taking anti-platelet agents within seven days of operation and those who had not (y/n, 681ml SD 131, 647ml SD 172, P_{Val} = 0.37) (table 4.5).

4.4.2.2 Time = ICU_{Ret} - 24h

The variables of; age (r = 0.21, P_{Val} = 0.28), height (r = 0.08, P_{Val} = 0.69), weight (r = 0.28, P_{Val} = 0.14), BSA (r = 0.27, P_{Val} = 0.16), initial patient RCV (r = 0.1, P_{Val} = 0.62), induction Hct (r = 0.13, P_{Val} = 0.52), CPB time (r = 0.13, P_{Val} = 0.52), ACC time (r = 0.12, P_{Val} = 0.54), minimum CPB temperature (r = 0.28, P_{Val} = 0.15), max re-warm temperature (r = 0.24, P_{Val} = 0.21), theatre RCV loss (r = 0.09, P_{Val} = 0.63) and fluid administered in theatre (r = 0.27, P_{Val} = 0.15) showed no significant association with RCV loss. Again, no significant difference was observed with gender (m = 217ml SD 100, f = 186ml SD 136, P_{Val} = 0.33) nor those taking anti-platelet agents within seven days of operation and those who had not (y/n, 226ml SD 123, 199ml SD 113, P_{Val} = 0.75) (table 4.6).

Table (4.5) *Correlation co-efficient (r) for study variables with RCV loss from t = 0 - 24h. RCV = red cell volume, P_{Val} = P value, BSA = body surface area and Hct = haematocrit.*

Variable	**Correlation Co-efficient (r)**	$\mathbf{P}_{Val}$
Age (y)	0.29	0.12
Height (m)	0.19	0.32
Weight (kg)	0.08	0.68
BSA (m^2)	0.10	0.60
Initial RCV (ml)	0.22	0.25
Induction Hct (%)	0.27	0.16

Table (4.6) *Correlation co-efficient (r) for study variables with RCV loss from t = ICU_{Ret} - 24h. RCV = red cell volume, P_{Val} = P value, BSA = body surface area, Hct = haematocrit, CPB = cardio-pulmonary bypass, ACC = aortic cross clamp and Op = operative.*

Variable	**Correlation Co-efficient (r)**	$\mathbf{P}_{Val}$
Age (y)	0.21	0.28
Height (m)	0.08	0.69
Weight (kg)	0.28	0.14
BSA (m^2)	0.27	0.16
Initial RCV (ml)	0.10	0.62
Induction Hct (%)	0.13	0.52
CPB Time (min)	0.13	0.52
ACC Time (min)	0.12	0.54
Min Temp (^{0}C)	0.28	0.15
Max Temp (^{0}C)	0.24	0.21
Op RCV Loss (ml)	0.09	0.63
Op Fluid (ml)	0.27	0.15

4.4.3 Factors Affecting Red Cell Gain

4.4.3.1 Time = 0 - 24h

The variables of; age (r = 0.46, P_{Val} = 0.01), height (r = -0.51, P_{Val} = 0.005), weight (r = -0.59, P_{Val} = 0.001), BSA (r = -0.6, P_{Val} = 0.001) induction Hct (r = -0.54, P_{Val} = 0.003), initial RCV (r = -0.6, P_{Val} = 0.001) and gender (m = 1.4u SD 1.4, f = 2.6u SD 0.9, P_{Val} = 0.03) were significantly associated with RCV gain. No such association was found with anti-platelet therapy (y/n, 1.8u SD 1.3, 2.1u SD 1.4, P_{Val} = 0.24) (table 4.7).

4.4.3.2 Time = ICU_{Ret} - 24h

The variables of; age (r = 0.40, P_{Val} = 0.03), weight (r = -0.45, P_{Val} = 0.02), BSA (r = -0.43, P_{Val} = 0.02), induction Hct (r = -0.37, P_{Val} = 0.05), and initial RCV (r = -0.42, P_{Val} = 0.05) were significantly associated with RCV gain. No such association was found with height (r = -0.24, P_{Val} = 0.22), gender (m = 1.4u SD 0.7, f = 2.0u SD 0.8, P_{Val} = 0.07), and anti-platelet therapy (y/n, 1.6u SD 1.0, 1.6u SD 1.3, P_{Val} = 0.32). The operative variables of; CPB time (r = -0.04, P_{Val} = 0.84), ACC (r = 0.06, P_{Val} = 0.77), minimum temperature (r = -0.31, P_{Val} = 0.10) maximum temperature (r = 0.15, P_{Val} = 0.45), theatre RCV loss (r = 0.22, P_{Val} = 0.26) and theatre fluid administration (r = 0.19, P_{Val} = 0.33) showed no significant association with RCV gain for this time period (table 4.8).

4.4.4 Association Between Red Cell Loss and Gain

No association was found between RCV gain and total RCV loss(ml) at t = 24h (r = 0.33, P_{Val} = 0.08). When RCV loss was expressed as percentage reduction in the initial estimated patient RCV a significant association with RCV gain was identified (r = 0.48, P_{Val} = 0.009).

Table (4.7) *Correlation co-efficient (r) for study variables with RCV Gain from t = 0 - 24h. RCV = red cell volume, P_{Val} = P value, BSA = body surface area and Hct = haematocrit.*

Variable	**Correlation Co-efficient (r)**	$\mathbf{P}_{Val}$
Age (y)	0.46	0.01
Height (m)	-0.51	0.005
Weight (kg)	-0.59	0.001
BSA (m^2)	-0.60	0.001
Initial RCV (ml)	-0.60	0.001
Induction Hct (%)	-0.54	0.003

Table (4.8) *Correlation co-efficient (r) for study variables with RCV gain from t = ICU_{Ret} - 24h. RCV = red cell volume, ICU = intensive care unit, P_{Val} = P value, BSA = body surface area, Hct = haematocrit, CPB = cardio-pulmonary bypass, ACC = aortic cross clamp and Op = operative.*

Variable	**Correlation Co-efficient (r)**	$\mathbf{P}_{Val}$
Age (y)	0.40	0.03
Height (m)	-0.24	0.22
Weight (kg)	-0.45	0.02
BSA (m^2)	-0.43	0.02
Initial RCV (ml)	-0.42	0.05
Induction Hct (%)	-0.37	0.05
CPB Time (min)	-0.04	0.84
ACC (min)	0.06	0.77
Min Temp (^{0}C)	-0.31	0.10
Max Temp (^{0}C)	0.15	0.45
Op RCV Loss (ml)	0.22	0.26
Op Fluid (ml)	0.19	0.33

4.5 Summary of Principle Findings

For both study time periods, no significant association with RCV loss was identified for the variables of; age, sex, height, weight,BSA, induction Hct, initial RCV, anti-platelet therapy, CPB time, ACC time, minimum and maximum temperature, theatre RCV loss and theatre clear fluid administration. When considering RCV gain, the pre-operative variables of; age, sex, height, weight, BSA, induction Hct, initial RCV were strongly correlated from t = 0 - 24h. This remained the case for t = ICU_{Ret}- 24h with the exception of height. Anti-platelet therapy showed no significant association for either time period. Similarly, the operative variables of; CPB time, ACC time, minimum and maximum temperature, theatre RCV loss and theatre clear fluid administration showed no significant correlation with RCV gain. Finally, it was found that RCV gain was associated with RCV loss only when expressed as percentage reduction in initial patient RCV.

Please see section 12.2 (page - 154) for a full discussion of the above findings.

Chapter 5

Peri-operative Red Cell, Plasma and Blood Volume Change

5.1 Background

As discussed in the previous chapter 4 (page - 53, pre-operative RCV may be an important factor in determining a patients requirement for RBC transfusion. Those patient's with a small RCV prior to operation have a lesser reserve to cope with RCV loss[137;173;174]. The next important consideration is to quantify the degree of haemodilution, primarily due to the extra-corporeal circuit, that patients undergo[127;128;139]. Currently, [Hb] is often the sole parameter considered when deciding whether to administer RBCs. This variable may be artificially depressed by the aforementioned fluid loading, triggering transfusion despite the loss of little RCV[129]. Therefore, considering estimates of pre-operative RCV and haemodilution, instead of or as well as [Hb], in the decision making process may identify those requiring RBCs while reducing the incidence of potentially unnecessary transfusion in others.

This study was designed to record peri-operative change in RCV as opposed to [Hb] or Hct. Estimates were also made of PV and BV from patient Hct.

5.2 Aim

To determine the relative contributions of RCV loss and haemodilution to the post-operative anemia encountered following cardiac surgery.

5.3 Materials and Methods

5.3.1 Patients

Please see section 4.3.1 (page - 55)*.

5.3.2 Patient Management

Please see section 4.3.2 (page - 56)*.

5.3.3 Red Cell Transfusion

Please see section 4.3.3 (page - 56).

5.3.4 Calculation of Red Cell Volume Loss and Gain

Please see section 4.3.4 (page - 57)*.

5.3.5 Calculation of Red Cell, Plasma and Blood Volume change

To estimate changes in patient RCV, the volume of RBCs lost or gained was added or subtracted from an equation derived initial volume for each study time point as outlined in section 4.3.4 (page - 57). This study used the formula for RCV calculation as recommended by the ICSH (equations A.2 and A.3)[171]. BSA was determined as recommended by Gehan and George (equation A.1)[170]. Both PV and BV were derived from the calculated RCV and the measured Hct (equations A.5 and A.4). Study time points were; induction (defined for the reporting of results as t = 0), incision, on-CPB, off-CPB, ICU_{Ret} and 1,3,6,10,16 and 24h following ICU_{Ret}.

*The patient cohort for this study was as for Chapter 4 (page - 53) with 1 additional patient. As an identical methodology was employed this section is as previously described.

5.3.6 Statistical Analysis

All variables were presented as mean (SD). A Student's t-test was used to determine the significance of any difference in each of the variables between two time points. A $P_{Val} \leq 0.05$ was considered statistically significant. SPSS version 13 was used for all statistical analysis.

5.4 Results

5.4.1 Demographic and Peri-operative Data

Thirty-three adult elective cardiac surgery patients were recruited to the study over a three month period. Three patients were subsequently excluded due to surgical re-exploration for bleeding. Of the thirty patients with complete data, 23 underwent CABG, 3 a AVR, 2 a MVR, 1 a CABG and MVR and 1 a AVR and MVR. The mean age was 63y (SD 13.0) with a BSA of 1.9m^2 (SD 0.3) and pre-operative Hct of 40.6% (SD 3.3). Eighteen of the patients were male, twelve female. During operation the mean CPB time was 90min (SD 31) with the ACC applied for 54min (SD 25). The minimal temperature on cooling was 31.7^{0}C (SD 1.6) with a max re-warm temperature of 37.2^{0}C (SD 0.3). Clear fluid administration from t = 0 - 24h was 2111ml (SD 897) for colloid and 6699ml (SD 1326)* for crystalloid (table 5.1).

*This figure includes the 2250ml extra-corporeal circuit prime.

Table (5.1) *Patient and operative characteristics. BSA = body surface area, Hct = haematocrit, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, MVR = mitral valve repair/replacement, CPB = cardio-pulmonary bypass and ACC = aortic cross clamp. All data is presented as mean (SD).*

Patient Variables (n = 30)	Age (y)	63 (13)
	Gender (m/f)	18/12
	BSA (m^2)	1.9 (0.3)
	Pre-operative Hct (%)	40.6 (3.3)
Operative Variables (n = 30)	CABG	23
	AVR	3
	MVR	2
	CABG + MVR	1
	AVR + MVR	1
	CPB Time (min)	90 (31)
	ACC Time (min)	54 (25)
	Min Temp (^{0}C)	31.7 (1.6)
	Max Temp (^{0}C)	37.2 (0.3)

5.4.2 Red Cell Volume Lost and Gained

The volume remaining in the extra-corporeal circuit accounted for the greatest RCV loss (391ml SD 115). It should be noted however, that a large proportion of this 'pump blood' was re-infused (195ml SD 90)*. Only 4 patients were transfused RBCs from t = 0 - ICU_{Ret}. For this time period, the net RCV balance was a loss of 213ml (SD 206) (table 5.2). From t = ICU_{Ret}- 24h the RCV lost gradually decreased giving a total of 200ml SD 116. The RCV gained for this period was in excess of this figure (274ml SD 200) giving a net gain for the period of 75ml (SD 198) (table 5.3). There was no significant difference noted in terms of RCV loss between the four surgeons taking part in the study.

*The full extra-corporeal circuit volume was considered lost at t = off-CPB as although RCV was re-infused it was not part of the patients circulating BV at this time point.

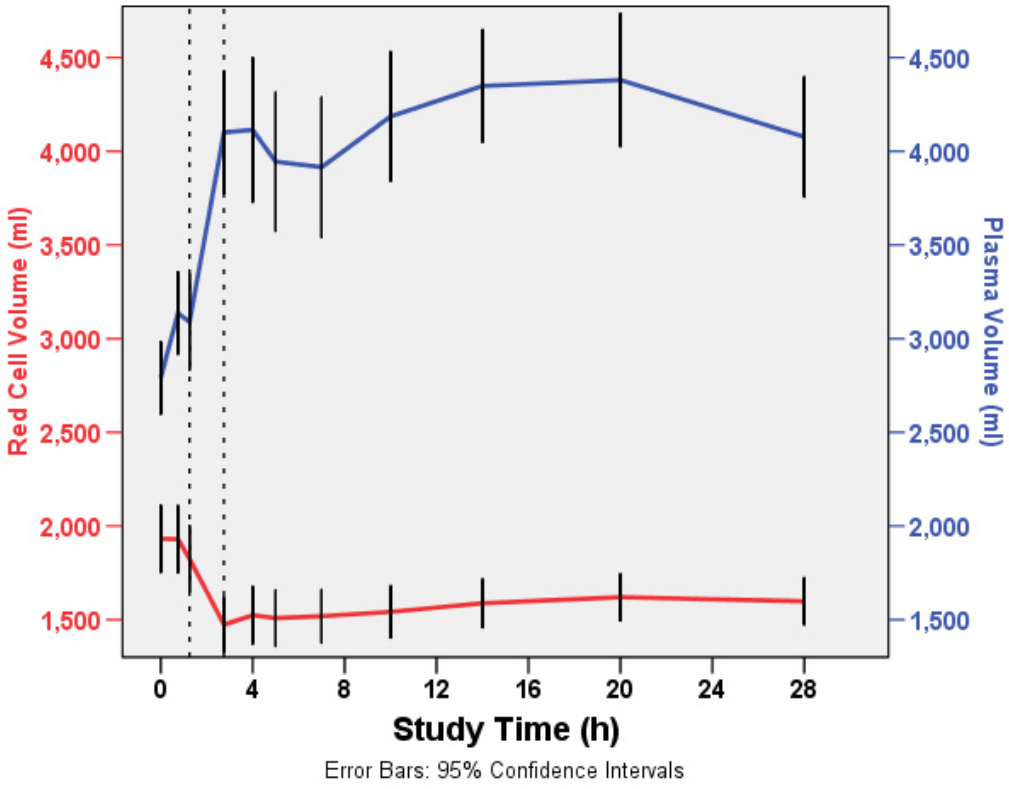
Table (5.2) *Theatre RCV loss and gain. RCV = red cell volume, Ind = induction, Inc = incision, ICU = intensive care unit return and CPB = cardiopulmonary bypass. All data is presented as mean (SD).*

RCV	**Ind-Inc**	**Inc-CPB**	**CPB**	**CPB-ICU$_{Ret}$**	**Total**
Loss (ml)	1	105	391	145	447
	(1)	(61)	(115)	(98)	(125)
Gain (ml)	0	0	39	195	234
			(105)	(90)	(133)
Net (ml)	-1	-105	-351	50	-213
	(1)	(61)	(163)	(142)	(206)

Table (5.3) *ICU RCV loss and gain, ICU = intensive care unit and RCV = red cell volume. All data is presented as mean (SD).*

RCV	**ICU$_{Ret}$-1h**	**1-3h**	**3-6h**	**6-10h**	**10-16h**	**16-24h**	**Total**
Loss (ml)	43	46	33	33	24	22	200
	(45)	(35)	(34)	(30)	(19)	(33)	(116)
Gain (ml)	28	56	56	78	56	0	274
	(78)	(92)	(92)	(106)	(92)		(200)
Net (ml)	-15	11	23	46	32	-22	75
	(83)	(102)	(91)	(113)	(92)	(33)	(198)

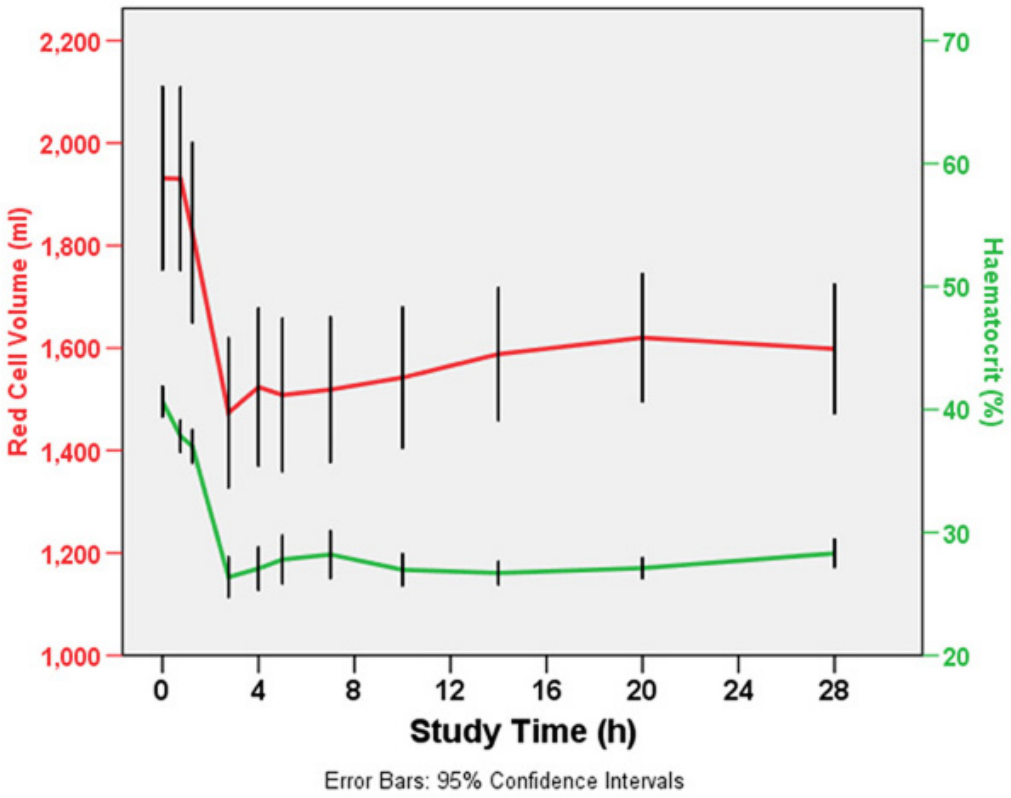
Figure (5.1) *RCV and PV for linear study time. Time = 0h equates to induction of anaesthesia while 4h is mean time for ICU return. Hashed lines indicate period of CPB. RCV = red cell volume, PV = plasma volume, ICU = intensive care unit and CPB = cardio-pulmonary bypass.*



5.4.3 Red Cell Volume Over Time

Calculated RCV (baseline plus or minus gains and losses) fell steadily from t = 0 - off-CPB (1931ml SD 479, 1474ml SD 391, P_{Val} = 0.63). At t = ICU_{Ret} a rise was noted due to the re-infusion of pump blood (1524ml SD 412, P_{Val} = 0.63). With RBC transfusion this trend continued until t = 16h (1620ml SD 334, P_{Val} = 0.32) (figure 5.1, tables 5.4 and 5.5). A comparison of change in patient RCV as compared to Hct is given in figure 5.2.

Figure (5.2) *RCV and Hct for linear study time. Time = 0h equates to induction of anaesthesia while 4h is mean time for ICU return. Hashed lines indicate period of CPB. RCV = red cell volume, Hct = haematocrit, ICU = intensive care unit and CPB = cardio-pulmonary bypass.*



5.4.4 Plasma Volume Over Time

Calculated PV rose from t = 0 - incision following the induction of general anaesthesia (2790ml SD 516, 3136ml SD 587, P_{Val} = 0.02). A further large increase was noted from t = on-CPB- off-CPB (3089 SD 683, 4101ml SD 873, $P_{Val} <$ 0.001). Thereafter a gradual fall occurred until until t = 3h (3915ml SD 1000, P_{Val} = 0.45) followed by a steady rise peaking at t = 16h (4380ml SD 951, P_{Val} = 0.07). At t = 24h a fall was noted (4077ml SD 858, P_{Val} = 0.2) (figure 5.1, tables 5.4 and 5.5).

Table (5.4) *Theatre RCV, PV and BV change. RCV = red cell volume, PV = plasma volume, BV = blood volume, Ind = induction, Inc = incision, CPB = cardio-pulmonary bypass and ICU = intensive care unit. All data is presented as mean (SD).*

	Ind	**Inc**	**On-CPB**	**Off-CPB**	**ICU**$_{Ret}$
RCV (ml)	1939	1930	1825	1474	1524
	(471)	(478)	(471)	(391)	(412)
PV (ml)	2790	3136	3089	4101	4114
	(516)	(587)	(683)	(873)	(103)
BV (ml)	4721	5076	4914	5575	5638
	(949)	(1003)	(1074)	(1139)	(1281)

5.4.5 Blood Volume Over Time

BV changes were similar to those for PV with a sharp rise noted from t = on-CPB- off-CPB (4914ml SD 1074, 5575ml SD 1139, $P_{Val} = 0.02$). Again, a fall occurred until t = 3h (5434ml SD 1224, $P_{Val} = 0.65$) with a subsequent rise noted at t = 16h (6000ml SD 1238) (tables 5.4 and 5.5).

Table (5.5) *ICU RCV, PV and BV Change. ICU = intensive care unit, RCV = red cell volume, PV = plasma volume and BV = blood volume. All data is presented as mean (SD).*

	1h	**3h**	**6h**	**10h**	**16h**	**24h**
RCV (ml)	1508	1519	1542	1588	1620	1598
	(401)	(380)	(369)	(347)	(334)	(338)
PV (ml)	3944	3915	4186	4348	4380	4077
	(992)	(1000)	(925)	(803)	(952)	(858)
BV (ml)	5452	5434	5728	5936	6000	5675
	(1229)	(1224)	(1217)	(1102)	(1238)	(1132)

Table (5.6) *Ratios of pre-operative values for RCV, adjusted RCV, PV and BV. RCV = red cell volume, RCV_{Adj} = RCV minus transfused RBCs, PV = plasma volume, BV = blood volume and ICU = intensive care unit. All data is presented as mean (SD).*

	Ind - $\mathbf{ICU_{Ret}}$	**$\mathbf{ICU_{Ret}}$ - 24h**
RCV	0.79 (0.99)	0.84 (0.10)
RCV_{Adj}	0.76 (0.07)	0.65 (0.25)
PV	1.48 (0.25)	1.47 (0.18)
BV	1.20 (0.17)	1.21 (0.11)

5.4.6 Ratios of Pre-operative Predicted Values for Red Cell, Plasma and Blood Volume at Closure and 24h

The ratios of the pre-operative predicted values for PV and BV showed marked increases both from t = 0 - ICU_{Ret} (1.48 SD 0.25, 1.20 SD 0.17) and t = 0 - 24h (1.47 SD 0.18, 1.21 SD 0.11). The difference between the RCV ratio and the value adjusted to exclude transfused RBCs was minimal at ICU_{Ret} (0.79 SD 0.99, 0.76 SD 0.07) but more marked at 24h (0.84 SD 0.10, 0.65 SD 0.25) (table 5.6).

5.5 Summary of Principle Findings

In terms of RCV loss, the maximal figure obtained was at t = off-CPB. The vast majority of this lost was attributable to the residual RCV remaining in the extra-corporeal circuit. Prior to t = ICU_{Ret}, approximately half of this RCV was returned to the patient in the form of pump blood. From t = ICU_{Ret}- 24h the trend was for a net gain of RCV due to the transfusion of RBCs. Both PV and BV had similar trends with two distinct peaks at t = off-CPB and 16h following ICU_{Ret}. Overall, at t = 24h the percentage rise in PV was greater than the percentage loss of RCV.

Please see section 12.3 (page - 156) for a full discussion of the above findings.

Chapter 6

Evaluation of Sodium Fluorescein Flow Cytometry in the Determination of Red Cell Volume

6.1 Background

As mentioned, the results gained in chapters 4 (page - 53) and 5 (page - 66) require to be treated with some caution due to the mechanical nature of the methodology used. In the past, direct measurement of RCV has relied upon the use of radioactive isotope labelled RBCs using elements such as such as Cr^{51} [175]. These techniques have the disadvantage that they require the use of ionising radiation and cannot be used over a short period. A method has been proposed whereby RCV may be measured by the use of autologous NaF labelled RBCs. The advantages of this technique are that it is non-toxic and repeat testing may be carried out after one hour [148;176].

This study was designed to assess NaF flow cytometry as an accurate reproducible method of measuring peri-operative RCV change. It was intended to perform this initial evaluation by the use of pre-operative isovolaemic haemodilution (PIHD) where a known volume of RCV is removed from the patient [130]. Unfortunately, the RBC-NaF complex was not sufficiently stable to allow us to proceed. Hence, only the techniques of NaF flow cytometry and PIHD will be described here along with the initial evaluation of NaF flow cytometry.

6.2 Aim

To assess the accuracy of NaF flow cytometry in the measurement of changes in RCV by PIHD.

6.3 Materials and Methods

6.3.1 Red Cell Volume Measurement by Sodium Fluorescein Flow Cytometry

The following summarises a version of the technique initially described by Lauermann et al for measuring RCV by NaF flow cytometry[130;148;176];

> Initially, 20ml of the patient's blood is collected in a heparinised syringe. The sample is then be spun at 1000g for 10min to separate out the RBC column. The RBCs obtained are incubated with 24mg of sodium fluorescein for five minutes and then washed twice with calcium containing solution. The RBCs are re-suspended to 20ml with Ringer's lactate solution. The resultant injectate is placed on ice prior to re-injection. Samples for analysis are taken at 4, 6 and 8min in duplicate, following re-injection. Samples aspirated are analysed on a Coulter FACS analyser with the fluorescent RBC population measured dependent upon light scattering characteristics.

The results obtained can be used to calculate RCV as outlined in appendix A.6 (page - 181).

6.3.2 Assessment of Sodium Fluorescein Flow Cytometry

PIHD is currently being used as a method of reducing allogenic RBC transfusion in cardiac surgery patients[115;142]. With this method a given volume of whole blood is withdrawn from the patient and replaced with an identical volume of plasma protein solution. Should transfusion be required then the patients own previously donated blood is re-infused. This technique was selected to evaluate NaF flow cytometry as the RCV within the PIHD bag can be directly measured[130].

NaF RCV estimation was to be performed prior to, and following PIHD with the change in RCV calculated (ΔRCV). This could then be compared with the known RCV content in the PIHD bag returned to the patient (equation A.7).

6.3.3 Statistical Analysis

When deciding upon sample size, it was considered that agreement between the two measurements was an estimation problem rather than a hypothesis testing issue. The planned study size of 30 patients was expected to result in a standard error of approximately 16ml for the 95% limits of agreement between the RCV removed during PIHD and change in RCV estimated using NaF, assuming a standard deviation for the difference between the methods of 50ml. A two group t-test was to be performed to determine the statistical significance of any observed difference between the two measurements. If the assumptions of the two group t-test were not met then non-parametric analysis would have been employed. A $P_{Val} \leq 0.05$ would be considered statistically significant. SPSS version 13 was used for all statistical analysis.

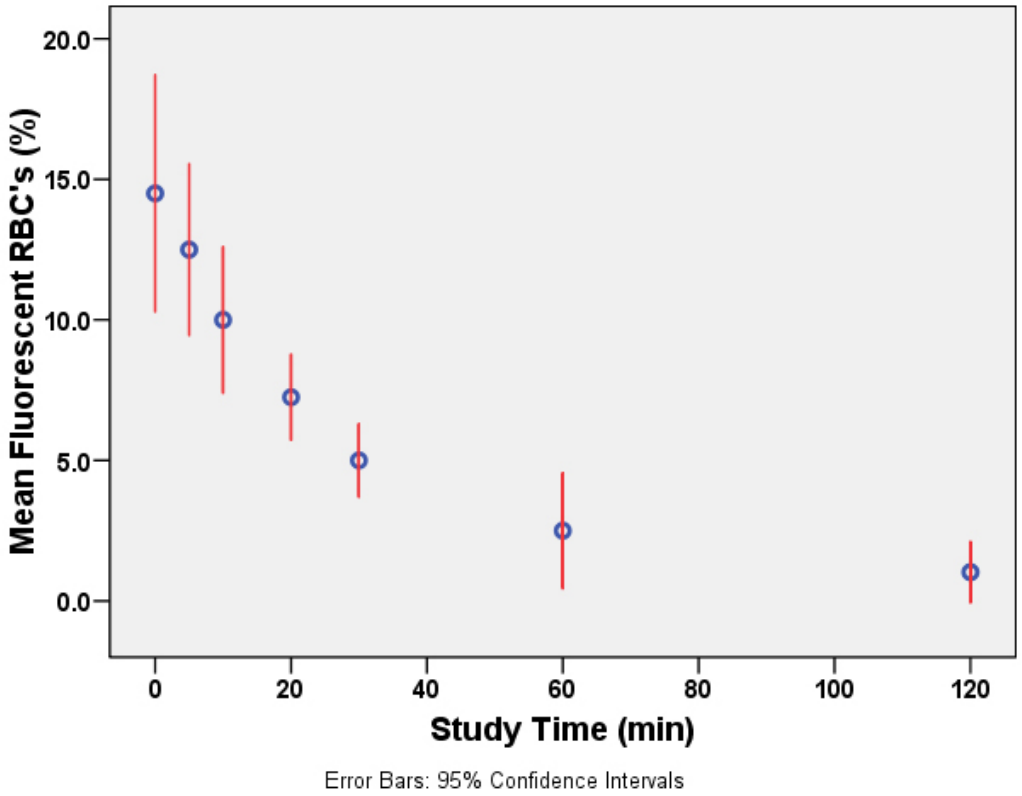
Table (6.1) *Reduction in NaF stained RBCs over time. NaF = sodium fluorescein and RBC = red blood cell.*

	Positive RBCs (%)				
Time (min)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Mean**
0	12	15	13	18	14.5
5	11	13	11	15	12.5
10	10	10	8	12	10.0
20	8	7	6	8	7.3
30	6	5	4	5	5.0
60	3	2	1	4	2.5
120	1	0.5	0.6	2	1.0

6.4 Results

Prior to commencing the study, as outlined above, 4 coulter FACS analyser* runs were performed for calibration purposes and to determine the stability of the RBC fluorescence. Following preparation of the RBC-NaF injectate, 250μl of the solution was added to 2.5ml of whole blood. Following thorough mixing, 10μl of the new suspension was then added to 500μl of ringers lactate solution. The result was an RBC sample containing approximately 15% fluorescent cells. Scans were then performed at; 0 (defined for the reporting of results as t = 0), 5, 10, 20, 30, 60 and 120min.

Figure (6.1) *Decay in NaF RBC's. NaF = sodium fluorescein and RBC = red blood cell.*



*FACS Calibur, Becton Dickinson, San Jose, USA.

The results obtained indicated a rapid decay in the % of NaF positive RBCs (table 6.1). From an initial value of 14.5% at t = 0, this had exactly halved by t = 20min (7.3%). By the final time point at t = 120min, the fluorescent cell population had fallen to a mere 1%, possibly due to enzymatic degradation of the stained RBCs amine groups. An error bar plot of the exponential decay in fluorescence is given in figure 6.1. This demonstrated a strong non-linear association between % fluorescent RBCs and time (r = 0.93, P_{Val} <0.001). In terms of practical application, these findings negated the clinical application of this process given the required timescales for injectate preparation and repeat measurement. A single measurement comparison with an equation derived RCV was not performed as it was felt this would not give the desired degree of accuracy achievable with more stable RBC labels.

6.5 Summary of Principle Findings

Prior to the evaluation of NaF flow cytometry, by PIHD, 4 FACS Calibur runs were performed with a 15% solution of NaF stained RBCs. By t = 20min, the fluorescence had exactly halved to a mean of 7.3%. By t = 120min the corresponding figure was 1%. The timescales involved prevented the clinical evaluation of this technique as intended.

Please see section 12.4 (page - 158) for a full discussion of the above findings.

Chapter 7

The Effect of Gain in Total Body Water on Haemoglobin Concentration and Body Weight

7.1 Background

As demonstrated in chapter 5 (page - 66), cardiac surgery is associated with a heavy fluid load, primarily due to the pump priming solution and the administration of intravenous fluids[38;127;139]. This may depress [Hb][129;177]. As most guidelines for RBC transfusion are based upon [Hb], this effect of haemodilution may result in RBCs being given despite the loss of little RCV.

Following cardiac surgery on CPB, patients are routinely prescribed a diuretic preparation in order to facilitate the shedding of excess total body water (TBW). A rise in [Hb] is routinely observed as excess TBW is lost. Therefore, consideration of patient fluid status, as well as [Hb], may reduce the incidence of RBC transfusion. Currently, fluid loss is monitored by recording daily patient body weight. As patients following cardiac surgery are in a highly catabolic state, this process may not be accurate[178].

This study was designed to examine the paired associations between; TBW (as measured by bio-electrical impedance analysis (BIA)), body weight and [Hb] following cardiac surgery.

7.2 Aim

To determine whether [Hb] will recover as excess TBW is shed post-operatively in association with a reduction in body weight.

7.3 Materials and Methods

7.3.1 Patients

Ethical approval was gained from LREC. All adult (age $\geq$ 16y) elective cardiac surgery patients presenting for operation between October and December 2004 were considered for recruitment. Exclusion criteria were; inability to provide informed consent, pre-operative anaemia ([Hb] $<$ 13g/dl for males and $<$ 11g/dl for females), RBC transfusion after 24h post-operatively, emergency surgery and redo surgery. In addition, patients with a pre-operative condition (thyroid or adrenal disease), or drug treatment (diuretics, steroids), interfering with total body fluid distribution were also excluded.

7.3.2 Patient Management

Please see section 4.3.2 (page - 56).

7.3.3 Diuretic Administration

On the first post-operative day all patients were prescribed a diuretic preparation according to routine practice (co-amilofruse (5mg Amiloride/40mg Frusemide)). When TBW had returned to its pre-operative state, as indicated by BIA measurements, this was discontinued.

7.3.4 Red Cell Transfusion

A transfusion threshold of 6g/dl was used during CPB, changing to 8g/dl for all off-CPB time points*. Where a patient received a RBC transfusion after 24h post-operatively they were excluded.

*This transfusion strategy was more restrictive than standard departmental policy in order to ensure maximal study completion.

Figure (7.1) *Bodystat Dualscan Bio-electrical Impedance Analyser.*

7.3.5 Study Variables

Study data was collected pre-operatively, one day prior to surgery (defined for the reporting of results as t = 0) and at days 1, 3, 5 and 10 (t = 1 - 10d) post-operatively. TBW was measured by the use of BIA as outlined below. Samples were also taken for [Hb], with body weight recorded on the same precision balances.

7.3.6 Bio-electrical Impedance Analysis

The conductive properties of tissues were first described in 1871. The 1970's saw the development of BIA, which today is widely used as a method of assessing nutritional status and body water volumes. Impedance describes the resistive force to electrical currents created by both cell membranes (reactance) and body water (resistance). Thus, the impedance of any tissue is determined by the tissue fluid content. Fat free mass (FFM) is highly hydrated and therefore a good conductor (low impedance), while poorly hydrated adipose tissue is a good insulator (high impedance). When low frequency signals (<50KHz) are applied, the current cannot penetrate the cell membrane and the impedance measured is limited to the extra-cellular fluid (ECF) compartment. High frequency signals (>50KHz), can traverse the cellular membrane and thus the impedance is generated by the TBW[38;127;179].

For the purposes of this study, the current injecting and current sensing electrodes of the Bodystat analyser were placed on the wrist and ankle respectively

(figure 7.1)*. Measurements were taken of the length^2/impedance index (L^2/I index) using high frequency signals (>50KHz). The L^2/I index measured under these conditions has been shown to be well correlated with TBW[179;180].

7.3.7 Statistical Analysis

All variables were presented as mean (SD). For the purposes of the analysis, all data was adjusted to reflect the percentage change from the baseline pre-operative value (t = 0). Student's t-test was used to determine the significance of the difference in the percentage change from t = 0 between two study time points. The percentage change in each of the variables was recorded from t = 0 - 1d, 1 - 3d, 1 - 5d, 1 - 10d and 5 - 10d. Pearson's rank correlation was performed to determine the paired associations between TBW and body weight, TBW and [Hb] and finally body weight and [Hb]. A $P_{Val} \leq 0.05$ was considered statistically significant. SPSS version 13 was used for all statistical analysis.

7.4 Results

7.4.1 Demographic and Peri-operative Data

Forty-two patients adult elective cardiac surgery patients were recruited to the study over a three month period. Twelve patients were subsequently excluded due to transfusion after 24h (n = 4) or missed follow-up appointments (n = 8). Of the thirty patients with complete data, 22 underwent CABG, 4 a AVR, 2 a CABG and AVR and 2 a atrial septal defect (ASD) repair. The mean age was 59.1y (SD 12.8) with a BSA of 2.1m^2 (SD 0.2) and pre-operative [Hb] of 14.2g/dl (SD 1.1). Twenty-five of the patients were male, five female. During operation the mean CPB time was 157min (SD 52.8) with the ACC applied for 99min (SD 41.8). The minimal temperature on cooling was 29.1^{0}C (SD 2.3) with a max re-warm temperature of 37.1^{0}C (SD 0.2) (table 7.1).

*Bodystat Dualscan, Bodystat Ltd, Isle of Man, UK.

Table (7.1) *Patient and operative characteristics. BSA = body surface area, Hb = haemoglobin, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, ASD = atrial septal defect, CPB = cardio-pulmonary bypass and ACC = aortic cross clamp. All data is presented as mean (SD).*

Patient Variables (n = 30)	Age (y)	59.1 (12.8)
	Gender (m/f)	25/5
	BSA (m^2)	2.1 (0.2)
	Pre-operative Hb (g/dl)	14.2 (1.1)
Operative Variables (n = 30)	CABG	22
	AVR	4
	CABG + AVR	2
	ASD	2
	CPB Time (min)	157 (52.8)
	ACC Time (min)	99 (41.8)
	Min Temp (^{0}C)	29.1 (2.3)
	Max Temp (^{0}C)	37.1 (0.2)

Table (7.2) *Peri-operative clear fluid and RBC transfusion. RBC = red blood cell. All data is presented as mean (SD).*

Intra-operative	Crystalloid (ml)	2768 (1123)
	Colloid (ml)	227 (410)
	Total Clear Fluids (ml)*	2996 (1160)
Intensive Care Unit	Crystalloid (ml)	1699 (681)
	Colloid (ml)	1600 (669)
	Total Clear Fluids (ml)	3299 (1119)
	Patients Transfused RBC's (%)	27 (n = 8/30)
	Mean RBC's per Patients Transfused (u)	2 (1.0)

* This figure includes the 2250ml extra-corporeal circuit prime.

Table (7.3) *Chest drain loss following operation. All data is presented as mean (SD).*

Time Period	**C**hest Drain Loss (ml)
0-6h	243 (115)
6-12h	175 (175)
Total at Removal	747 (444)

A total of 2996ml (SD 1160)* of clear fluid (crystalloid and colloid) was administered during theatre management. The corresponding figure for the period of ICU management until t = 1d was 3299ml (SD 1119). No patients were transfused intra-operatively. During ICU management, 27% of patients were transfused a mean of 2u of RBCs (table 7.2). The mean drain volume loss for the first 6h following surgery was 243ml (SD 115). This fell to 175ml (SD 73) for the second 6h with a total at removal of 747ml (SD 444). The mean time until drain removal was 20.4h (SD 7.1) (table 7.3).

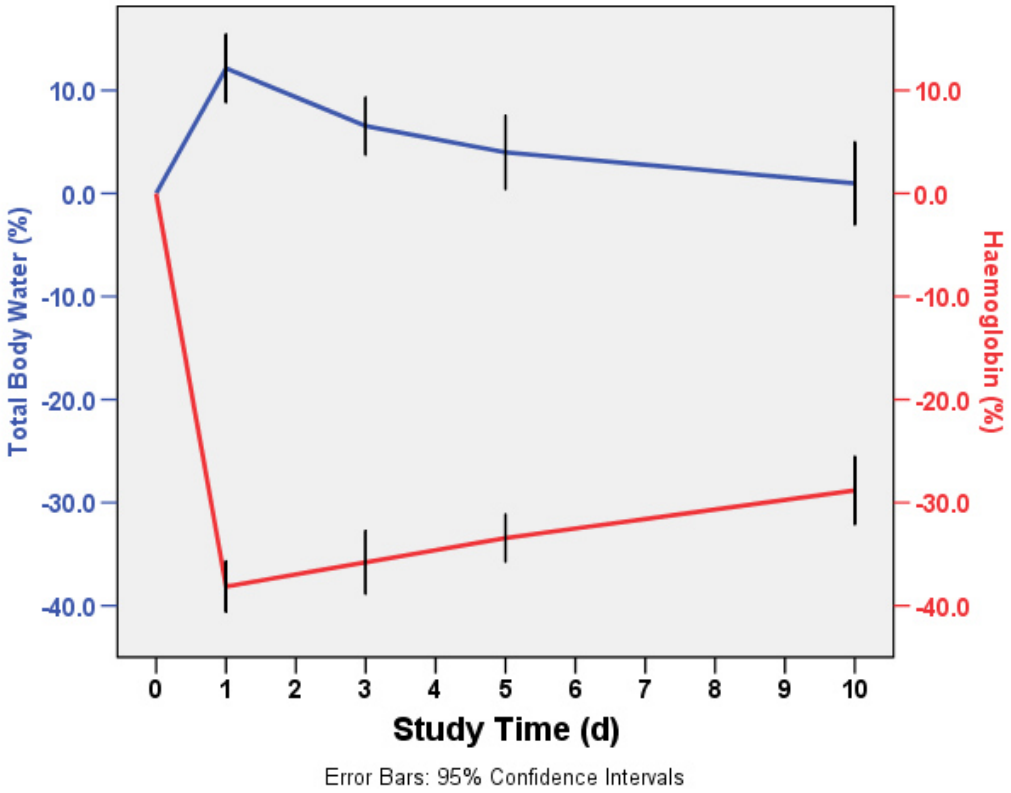
7.4.2 Total Body Water, Body Weight and Haemoglobin over Time

A sharp fall in TBW was noted between t = 1 - 3d (%Δ12.1 SD 8.8, %Δ6.5 SD 7.5, $P_{Val} < 0.001$) with this being the overall trend from t = 1 - 10d (%Δ12.1 SD 8.8, %Δ1.0 SD 10.7, $P_{Val} < 0.001$). At t = 10d, TBW approximated the baseline pre-operative value (43.8l SD 9.0, 43.4l SD 7.8, $P_{Val} = 0.77$). A similar pattern was noted for body weight with a sharp fall between t = 1 - 3d (%Δ4.7 SD 3.3, %Δ1.8 SD 2.5, $P_{Val} < 0.001$) and a significant overall loss demonstrated from t = 1 - 10d (%Δ4.7 SD 3.3, %Δ5.2 SD 3.8, $P_{Val} < 0.001$). At t = 10d, the value had fallen below that measured pre-operatively (87.3kg SD 14.5, 86.9kg SD 15) although this was not significant ($P_{Val} = 0.31$) (figures 7.2, 7.3 and table 7.4).

A steady recovery in [Hb] was from t = 1 - 10d (%Δ-37.0 SD 6.4, %Δ-27.6 SD 8.3, $P_{Val} < 0.001$), the biggest rise occurring between t = 5 - 10d (%Δ-32.2 SD

*This figure includes the 2250ml extra-corporeal circuit prime.

Figure (7.2) *Percentage change in TBW and Hb. TBW = total body water and Hb = haemoglobin concentration.*

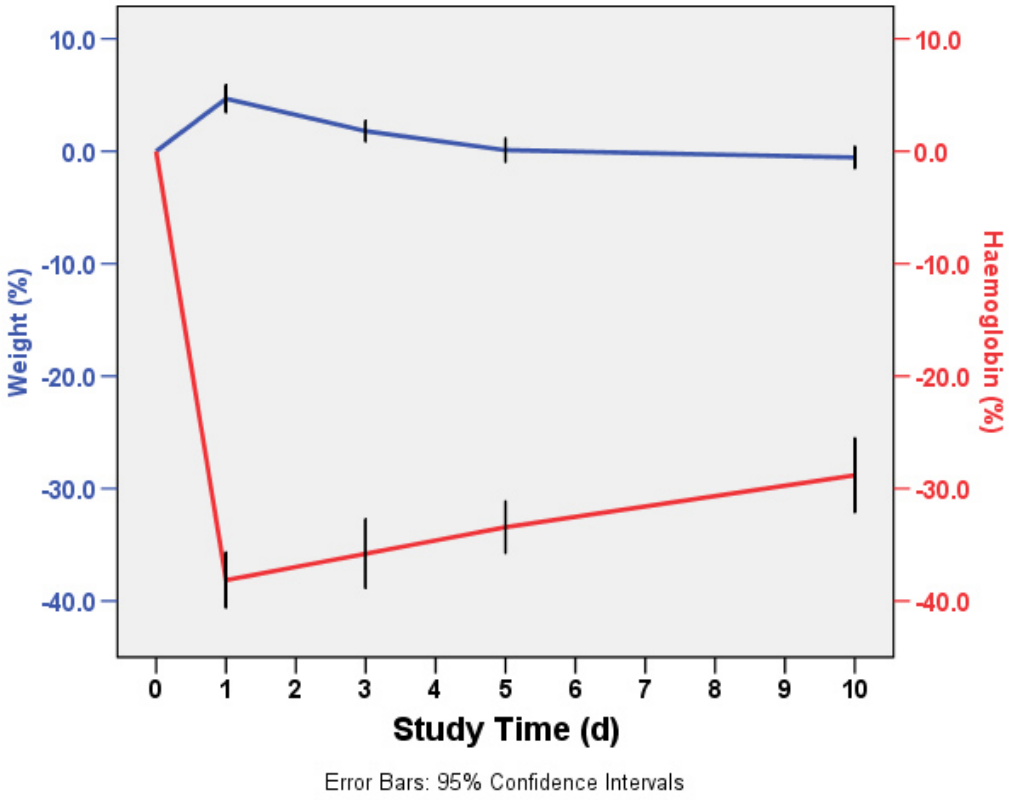


5.9, %Δ-27.6 SD 8.3, $P_{Val} = 0.001$). At t = 10d the value remained significantly below that recorded pre-operatively (10.2g/dl SD 1.1, 14.2g/dl SD 1.2, $P_{Val} < 0.001$) (figures 7.2, 7.3 and table 7.4).

7.4.3 The Association between Change in Total Body Water, Body Weight and Haemoglobin Concentration

Strong association was identified between change in TBW & body weight at t = 0 - 1d (r = 0.51, $P_{Val} = 0.004$). For the same period, no such association was found between TBW & [Hb] (r = -0.21, $P_{Val} = 0.28$) or body weight & [Hb] (r = 0.05, $P_{Val} = 0.79$). At t = 1 - 5d, significant associations were identified between TBW & body weight (r = 0.55, $P_{Val} = 0.002$), TBW & [Hb] (r = -0.42, $P_{Val} =$

Figure (7.3) *Percentage change in body weight and Hb. Hb = haemoglobin concentration.*



0.02) and body weight & [Hb] (r = -0.37, P_{Val} = 0.04). This was also the case for TBW & body weight (r = 0.60, $P_{Val} < 0.001$) and TBW & [Hb] (r = -0.36, P_{Val} = 0.048) at t = 1 - 3d, however the association between body weight & [Hb] was non-significant for this time period (r = -0.33, P_{Val} = 0.07) (table 7.5).

When considering t = 1 - 10d and t = 5 - 10d, TBW & body weight demonstrated significant association for both periods (r = 0.43, P_{Val} = 0.02 and r = 0.39, P_{Val} = 0.04). Although there was significant association between TBW & [Hb] at t = 1 - 10d (r = -0.55, P_{Val} = 0.002), this was not found for the second half of this time period at t = 5 - 10d (r = -0.14, P_{Val} = 0.47). Between body weight & [Hb] no association was found at t = 1 - 10d (r = -0.32, P_{Val} = 0.09) and t = 5 - 10d (r = -0.23, P_{Val} = 0.23) (table 7.5).

Table (7.4) *TBW, body weight and Hb for study time points. TBW = total body water, Hb = haemoglobin concentration and %Δ = percentage change from the baseline pre-operative value. All data is presented as mean (SD).*

	TBW		**Body Weight**		**Hb**	
	l	**%Δ**	**kg**	**%Δ**	**g/dl**	**%Δ**
Pre-operative	43.4		87.3		14.2	
	(7.8)		(14.5)		(1.2)	
Day 1	48.8	12.1	91.4	4.7	8.9	-37.0
	(9.8)	(8.8)	(15.5)	(3.3)	(0.6)	(6.4)
Day 3	46.3	6.5	88.9	1.8	9.2	-34.6
	(8.9)	(7.5)	(14.7)	(2.5)	(0.8)	(8.2)
Day 5	45.0	4.0	87.4	0.1	9.6	-32.2
	(8.4)	(9.6)	(14.4)	(2.8)	(0.7)	(5.9)
Day 10	43.8	1.0	86.9	-0.5	10.2	-27.6
	(9.0)	(10.7)	(15.0)	(2.6)	(1.1)	(8.3)

Table (7.5) *Correlation co-efficient (r) for percentage change in study variables. TBW = total body water, Wt = body weight, Hb = haemoglobin concentration and P_{Val} = P value.*

	TBW & Wt		**TBW & Hb**		**Wt & Hb**	
	R	**P_{Val}**	**R**	**P_{Val}**	**R**	**P_{Val}**
T = 0 - 1d	0.51	0.004	-0.21	0.28	0.05	0.79
T = 1 - 3d	0.60	<0.001	-0.36	0.05	-0.33	0.07
T = 1 - 5d	0.55	0.002	-0.42	0.02	-0.37	0.04
T = 1 - 10d	0.43	0.02	-0.55	0.002	-0.32	0.09
T = 5 - 10d	0.39	0.04	-0.14	0.47	-0.23	0.23

7.5 Summary of Principle Findings

The findings of this study have demonstrated a sharp associated rise in TBW & body weight between t = 0 - 1d. [Hb] fell for the same period, however this was not associated with either variable. Following this time point (t = 1 - 10d), an associated fall occurred between TBW & body weight. At t = 5d bodyweight approximated its pre-operative value. This did not occur with TBW until t = 10d. [Hb] recovered in association with TBW until t = 5d. A similar association was found with body weight and [Hb] for the same time period (t = 1 - 5d).

Please see section 12.5 (page - 159) for a full discussion of the above findings.

Chapter 8

Oxygen Delivery and Haemoglobin Concentration in Cardiac Surgery

8.1 Background

It has long been thought that patients undergoing cardiac surgery with the use of CPB accrue an oxygen debt peri-operatively[181;182]. Most commonly, this is believed to manifest itself as a post-operative metabolic acidosis[181]. One of the key observations supporting the debt theory has been so called "pathological supply dependency"where DO_2 determines VO_2 below a critical point (figure 1.7). Recently it has been recognised that this association is likely to represent mathematical coupling as opposed to a true patho-physiological state[92;93]. It has also been shown that the invariably encountered post-operative metabolic acidosis may accumulate under conditions of aerobic metabolism[99]. For many years, the oxygen debt theory has been used as a mainstay in the argument for maintaining a higher peri-operative [Hb] by the transfusion RBCs[25].

This study has been designed to use measurements of DO_2 to determine if patients approach an estimated DO_{2Crit} at any point during their peri-operative management.

8.2 Aim

To examine the relationship between actual measured oxygen delivery and the corresponding estimated critical values peri-operatively.

8.3 Materials and Methods

8.3.1 Patients

Ethical approval was gained from LREC. All adult (age $\geq$ 16y) elective cardiac surgery patients presenting for operation between May and July 2005 were considered for recruitment. Exclusion criteria were; inability to provide informed consent, renal insufficiency, diabetes mellitus, thyroid disease, severe hypertension, active endocarditis, heart failure and redo surgery.

8.3.2 Patient Management

Please see section 4.3.2 (page - 56).

8.3.3 Red Cell Transfusion

A transfusion threshold of 6g/dl was used during CPB, changing to 8g/dl for all off-CPB time points.

8.3.4 Study Variables

This study was based upon measurements of DO_2 at several time points perioperatively. Measured actual values were compared against estimated critical values corrected for temperature (equations B.1 and B.3). The [Hb] reflecting the estimated DO_{2Crit} (critical haemoglobin concentration ($[Hb_{Crit}]$))(assuming other parameters are constant) was also derived.

The following time points were identified for the collection of study data; induction of anaesthesia (defined for the reporting of results as t = 0), 10min CPB, 30min CPB, 60min CPB, max re-warm, 5min post-protamine, ICU_{Ret}, 1,5,10 and 18h post ICU_{Ret}. At these intervals the following measurements/ samples were taken; blood flow index (BFI)*, arterial blood sample, mixed venous blood sample

*The term BFI (blood flow/BSA) was chosen as it applies to both the CPB and cardiac based circulation. It may be used interchangeably with CI (CO/BSA) for all non-CPB time points

and core body temperature (nasal temperature probe). It should be noted that BFI was constant during the period of CPB.

For all off-CPB time points, BFI was measured by the thermo-dilution technique using a Swann-Ganz catheter. During CPB, pump flow rate was divided by BSA to give this value. Mixed venous blood was obtained from the distal port of the Swann-Ganz catheter. Arterial blood was collected from the radial artery catheter. All blood sample analysis was performed on a Rapidlab 855 analyser (figure 4.1)*.

When considering DO_{2Crit}, perhaps the most rigorous study was performed by Ronco et al on 9 non-septic, critically ill, intensive care patients. VO_2, as measured by indirect calorimetry, was plotted against DO_2, as calculated by the Fick method, QO_2 and measurements of plasma lactate concentration (equations B.5 and B.6). DO_{2Crit} was found to occur at a value of 4.5ml/min/kg[97;183]. This, however, is not directly applicable to cardiac surgery patients who undergo a degree of controlled hypothermia. A correction coefficient was thus used to adjust the DO_{2Crit} for temperature (equation B.4)[98].

To provide an estimate of the [Hb] that would reflect DO_{2Crit} ($[Hb_{Crit}]$), DO_{2Crit} was substituted for actual DO_2 (equations B.1 and B.3) and $[Hb_{Crit}]$ derived. In performing this calculation the assumption was made that all other parameters would remain constant. As blood flow rate during CPB is relatively fixed, this scenario is directly applicable. As it may be reasonably expected that cardiac output would rise in response to such anaemia post-CPB, it is likely that the value for $[Hb_{Crit}]$ reflects the upper end of the critical range[184].

8.3.5 Statistical Analysis

All variables were presented as mean (SEM). A Mann-Whitney U test was used to determine the significance of any difference between the actual and critical values for both DO_2 and [Hb]. Wilcoxon's rank sum analysis was performed to determine the significance of any difference for a single variable between two study time points. A $P_{Val} \leq 0.05$ was considered statistically significant. SPSS version 13 was used for all statistical analysis.

*Bayer HealthCare, Leverkusen, Germany.

Table (8.1) *Patient and operative characteristics. BSA = body surface area, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, MVR = mitral valve replacement, CPB = cardio-pulmonary bypass and ACC = aortic cross clamp. All data is presented as mean (SEM) where appropriate.*

Patient Variables (n = 15)	Age (y)	66.9 (2.8)
	Gender (m/f)	9/6
	BSA (m^2)	1.9 (0.1)
Operative Variables (n = 15)	CABG	4
	AVR	6
	MVR	1
	CABG + AVR	1
	CABG + MVR	3
	CPB Time (min)	113 (9.6)
	ACC Time (min)	80 (7.6)
	Min Temp (^{0}C)	30.9 (0.1)
	Max Temp (^{0}C)	37.0 (0.5)

8.4 Results

8.4.1 Demographic and Peri-operative Data

Nineteen adult elective cardiac surgery patients were recruited to the study over a three month period. Four patients were subsequently excluded due to due to incomplete data recording. Of the fifteen patients with complete data, 4 underwent CABG, 6 a AVR, 1 a MVR, 1 a CABG and AVR and 3 a CABG and MVR. The mean age was 66.9y (SEM 2.8)with a BSA of 1.9m^2 (SEM 0.1). Nine of the patients were male, six female. During operation the mean CPB time was 113min (SEM 9.6) with the ACC applied for 80min (SEM 7.6). The minimal temperature on cooling was 30.1^{0}C (SEM 0.1) with a max re-warm temperature of 37.0^{0}C (SEM 0.5) (table 8.1).

Table (8.2) *Core body temperature and BFI. BFI = blood flow index, CPB = cardio-pulmonary bypass and ICU = intensive care unit. All data is presented as mean (SEM).*

Study Time Point	Core Temperature (^{0}C)	BFI (l/min/m^2)
Induction	35.8 (0.2)	2.2 (0.13)
10min CPB	32.2 (0.6)	2.4 (0.05)
30min CPB	31.5 (0.4)	2.4 (0.04)
60min CPB	30.1 (0.8)	2.2 (0.63)
Max Re-warm	37.0 (0.1)	2.3 (0.36)
Post-protamine	36.0 (0.1)	2.7 (0.12)
ICU$_{Ret}$	35.7 (0.1)	2.6 (0.14)
1h ICU	35.8 (0.2)	2.4(0.15)
5h ICU	37.1 (0.3)	2.9 (0.16)
10h ICU	37.8 (0.2)	3.1 (0.13)
18h ICU	37.3 (0.2)	3.2 (0.10)

8.4.2 Core Body Temperature and Blood Flow Index

From t = 0 - 10min CPB, systemic cooling significantly lowered core body temperature (35.8^{0}C SEM 0.2, 32.2^{0}C SEM 0.6, P$_{Val}$ = 0.001). This was reversed from t = 60min CPB- max re-warm (30.1^{0}C SEM 0.8, 37.0^{0}C SEM 0.1, P$_{Val}$ = 0.005), prior to a fall noted at t = ICU$_{Ret}$ (35.7^{0}C SEM 0.1, P$_{Val}$ = 0.001). The highest core body temperature occurred at t = 10h ICU, having rising steadily from t = ICU$_{Ret}$ (37.8^{0}C SEM 0.2, P$_{Val}$ = 0.001) (table 8.2).

BFI did not change significantly from t = 0 - 10min CPB (2.2l/min/m^2 SEM 0.14, 2.4l/min/m^2 SEM 0.05, P$_{Val}$ = 0.268). Only two significant changes did occur. The first between t = max re-warm - post-protamine, corresponding with the discontinuation of CPB (2.3l/min/m^2 SEM 0.36, 2.7l/min/m^2 SEM 0.12, P$_{Val}$ = 0.03). A second steady rise occurred between t = 1h - 10h ICU (2.4l/min/m^2 SEM 0.15, 3.2l/min/m^2 SEM 0.10, P$_{Val}$ = 0.001) (table 8.2, figure 8.2).

Table (8.3) *Observed and critical values for DO_2. DO_2 = oxygen delivery, P_{Val} = P value, CPB = cardio-pulmonary bypass and ICU = intensive care unit. All data is presented as mean (SEM).*

Study Time Point	**DO_2 (ml/min/m^2)**	**DO_{2Crit} (ml/min/m^2)**	**P_{Val}**
Induction	388 (27)	165 (4.9)	<0.001
10min CPB	271 (12)	116 (5.1)	<0.001
30min CPB	276 (13)	111 (4.3)	<0.001
60min CPB	325 (16)	117 (9.2)	<0.001
Max Re-warm	269 (15)	181 (5.6)	<0.001
Post-protamine	333 (19)	167 (3.6)	<0.001
ICU_{Ret}	340 (24)	164 (4.6)	<0.001
1h ICU	321 (19)	165 (5.1)	<0.001
5h ICU	338 (23)	185 (7.6)	<0.001
10h ICU	377 (17)	196 (7.2)	<0.001
18h ICU	386 (14)	188 (6.8)	<0.001

8.4.3 Oxygen Delivery and Consumption

When considering observed DO_2, a marked fall occurred between t = 0 - 10min CPB corresponding with the start of CPB and systemic haemodilution (388ml/min/m^2 SEM 27, 271ml/min/m^2 SEM 12, $P_{Val} = 0.001$). Thereafter, no significant change was noted in DO_2 until t = max re-warm - post-protamine where the heart resumed circulatory responsibility (269ml/min/m^2 SEM 15, 333ml/min/m^2 SEM 19, $P_{Val} = 0.008$). From t = ICU_{Ret}- 1h ICU a short fall occurred (340ml/min/m^2 SEM 24, 321ml/min/m^2 SEM 19, $P_{Val} = 0.16$) prior to a linear rise up until the final time point at t = 18h ICU (386ml/min/m^2 SEM 14, $P_{Val} = 0.002$) (figures 8.1, 8.2 and table 8.3).

As DO_{2Crit} was determined by temperature (equation B.4), peri-operative change mirrored that for core body temperature as described previously (section 8.4.2). At no study time point did observed DO_2 significantly approach DO_{2Crit} ($P_{Val} < 0.001$ at all times) (figure 8.1 and table 8.3).

Figure (8.1) *Observed and critical DO_2 for linear study time. Time = 0h equates to induction of anaesthesia while 4h is mean time for ICU return. Hashed lines indicate period of CPB. ICU = intensive care unit and CPB = cardio-pulmonary bypass.*

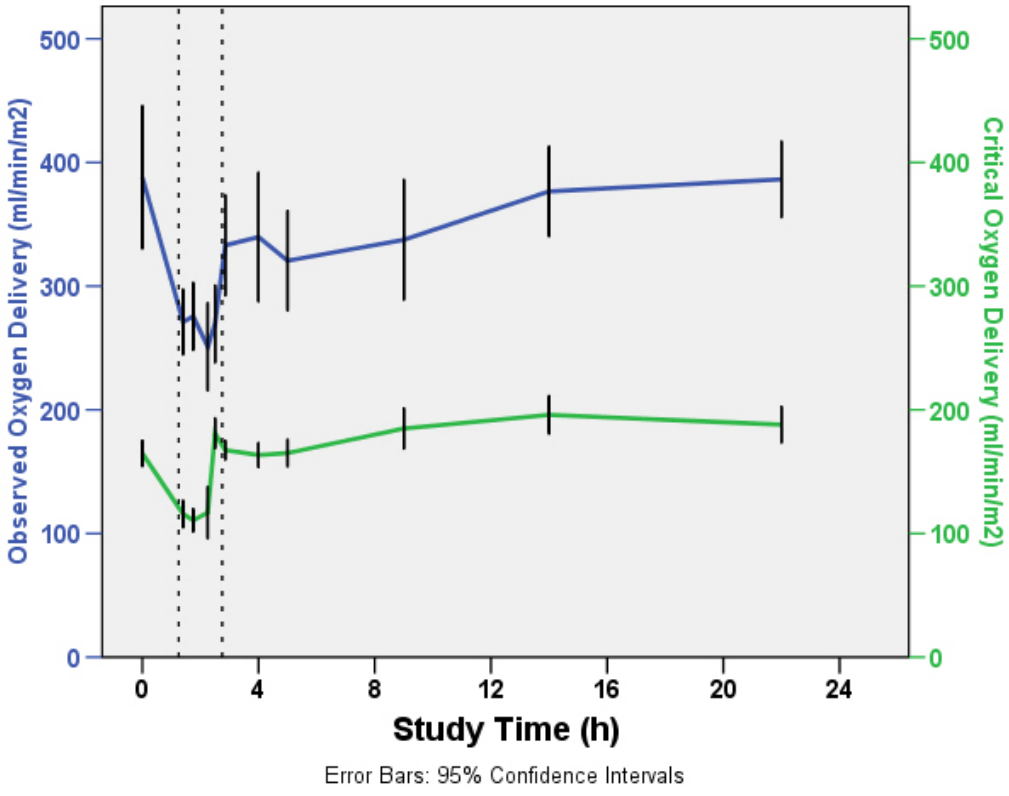


Figure (8.2) *Observed DO_2 and core body temperature for linear study time. Time = 0h equates to induction of anaesthesia while 4h is mean time for ICU return. Hashed lines indicate period of CPB. ICU = intensive care unit and CPB = cardio-pulmonary bypass.*

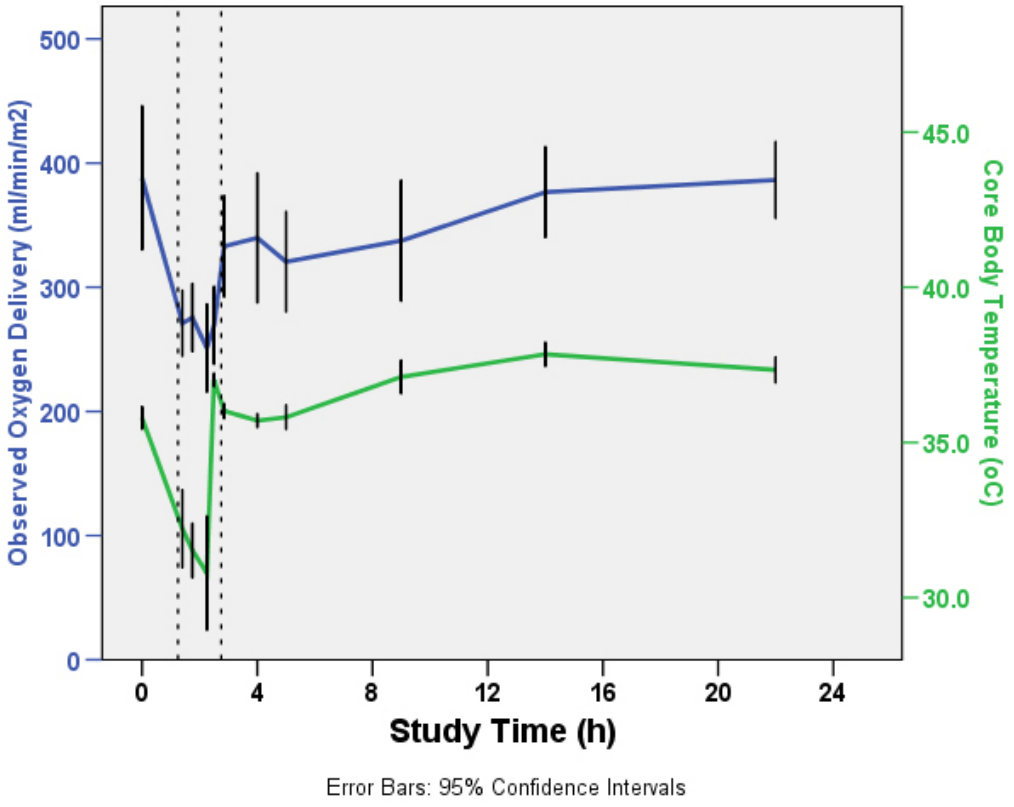


Table (8.4) *Peri-operative VO_2. VO_2 = oxygen consumption, CPB = cardio-pulmonary bypass and ICU = intensive care unit. All data is presented as mean (SEM).*

Study Time Point	**VO_2 ($ml/min/m^2$)**	**Study Time Point**	**VO_2 ($ml/min/m^2$)**
Induction	89 (6.6)	ICU_{Ret}	106 (4.8)
10min CPB	40 (3.9)	1h ICU	110 (4.0)
30min CPB	35 (4.3)	5h ICU	122 (7.3)
60min CPB	37 (3.1)	10h ICU	140 (7.4)
Max Re-warm	68 (4.2)	18h ICU	145 (7.7)
Post Protamine	86 (6.8)		

Table (8.5) *Observed and critical values for Hb. Hb = haemoglobin concentration, P_{Val} = P value, CPB = cardio-pulmonary bypass and ICU = intensive care unit. All data is presented as mean (SEM).*

Study Time Point	**Hb (g/dl)**	**Hb_{Crit} (g/dl)**	**P_{Val}**
Induction	12.9 (0.5)	5.8 (0.4)	<0.001
10min CPB	8.4 (0.4)	3.6 (0.2)	<0.001
30min CPB	8.6 (0.4)	3.5 (0.1)	<0.001
60min CPB	8.6 (0.5)	3.9 (0.2)	<0.001
Max Re-warm	8.6 (0.5)	5.8 (0.3)	<0.001
Post Protamine	9.2 (0.4)	4.7 (0.2)	<0.001
ICU_{Ret}	9.6 (0.5)	4.8 (0.3)	<0.001
1h ICU	10.2 (0.5)	5.4 (0.4)	<0.001
5h ICU	8.7 (0.5)	5.0 (0.3)	<0.001
10h ICU	9.1 (0.4)	4.8 (0.2)	<0.001
18h ICU	9.0 (0.3)	4.4 (0.2)	<0.001

Changes in VO_2 were similar to that for DO_2 with one exception. Again a fall occurred between t = 0 - 10min CPB (89ml/min/m^2 SEM 6.6, 40ml/min/m^2 SEM 3.9, P_{Val} = 0.001), thereafter remaining relatively constant during the period of CPB. As opposed to DO_2 however, VO_2 began its rise following the initiation of re-warming from t = 60min CPB- max re-warm (37ml/min/m^2 SEM 3.1 - 68ml/min/m^2 SEM 4.2, P_{Val} = 0.009). A further increase was noted from t = 1h - 18h ICU (110ml/min/m^2 SEM 4.0, 145ml/min/m^2 SEM 7.7, P_{Val} = 0.03) before leveling off (table 8.4).

8.4.4 Observed and Critical Haemoglobin Concentration

Between t = 0 - 10min CPB, actual [Hb] fell sharply, coinciding with the dilutional effect of commencing CPB (12.9g/dl SEM 0.5, 8.4g/dl SEM 0.4, P_{Val} = 0.001)[38;127;129;139;177]. Little variation in [Hb] was noted during CPB. Thereafter, a steady rise occurred from t = post-protamine - 1h ICU (9.2g/dl SEM 0.4 to 10.2g/dl SEM 0.5, P_{Val} = 0.021) followed by a significant fall to t = 5h ICU (8.7g/dl SEM 0.5, p = 0.004). After t = 5h ICU [Hb] remained relatively stable (table 8.5).

[Hb_{Crit}] similarly fell between t = 0 - 10min CPB (5.8g/dl SEM 0.4, 3.6g/dl SEM 0.2, P_{Val} = 0.001), again remaining relatively constant during CPB. With rise in core temperature and VO_2 between t = 60min CPB- max re-warm, [Hb_{Crit}] also rose (3.9g/dl SEM 0.2, 5.8g/dl SEM 0.3, P_{Val} = 0.007). It was at this point [Hb_{Crit}] reached its closest approximation to observed [Hb]. Post-operatively, [Hb_{Crit}] fell steadily between t = 1 - 18h ICU (5.4g/dl SEM 0.4, 4.4g/dl SEM 0.2, P_{Val} = 0.014). At no study time point did actual [Hb] significantly approach [Hb_{Crit}] ($P_{Val} < 0.001$ at all times) (table 8.5).

8.5 Summary of Principle Findings

Following the initiation of CPB the variables of; core body temperature, DO_2, DO_{2Crit}, VO_2, [Hb] and [Hb_{Crit}] all underwent a significant fall to a plateau and rose with re-warming. BFI underwent little change during the transition from cardiac to CPB based circulation. DO_2 and [Hb] reached their closest approximation with the corresponding critical values at t = max re-warm. The cessation of CPB, with restoration of the cardiac based circulation, brought about a rise in BFI. During the post-operative period, core body temperature, BFI, DO_2, DO_{2Crit} and [Hb] all fell initially followed by a linear rise towards the final study time point at t = 18h ICU. [Hb_{Crit}] fell steadily post-operatively from t = 1 - 18h ICU accompanied by a rise in VO_2. At all time points a statistically significant difference existed between observed DO_2, observed [Hb] and their corresponding critical values.

Please see section 8.5 (page - 105) for a full discussion of the above findings.

Chapter 9

The Impact of a Red Cell Volume Based Transfusion Guideline on Blood Usage and Clinical Outcome

9.1 Background

As mentioned in chapters 4 (page - 53),5 (page - 66) and 7 (page - 83), cardiac surgery, using CPB, is associated with profound haemodilution[38;127;129;139;177]. Use of [Hb] as a 'trigger' for RBC transfusion means that RBCs may be transfused despite the loss of little RCV[129;177]. The ultimate ambition in peri-operative transfusion medicine would be a simple reproducible test to accurately measure and replace RCV where required. Unfortunately, as mentioned in chapter 6 (page - 77), this is not possible with our current level of knowledge[148;176]. Thus, any attempt to base RBC transfusion on RCV can only be performed by an approximation derived from the patient and operative characteristics described previously (chapter 4, page - 53).

This study was designed to analyse the impact of a simple RCV based transfusion algorithm, derived from the aforementioned variables, on blood usage and clinical outcome.

9.2 Aim

To determine if RBC transfusion based upon an estimate of patient RCV confers benefit in terms of a reduction in blood usage and improved clinical outcome.

9.3 Materials and Methods

9.4 Patients

Ethical approval was gained from LREC. All adult (age $\geq$ 16y) elective cardiac surgery patients presenting for operation between June 2005 and May 2006 were considered for recruitment. Pre-operative exclusion criteria were; the inability to provide informed consent, emergency surgery, redo surgery, active endocarditis and poor left ventricular function (ejection fraction $<$ 30%). Any patient sustaining an intra-operative MI was also excluded.

9.4.1 Patient Management

Please see section 4.3.2 (page - 4.3.2).

9.4.2 Red Cell Transfusion

Prior to surgery patients were randomised (non-blinded) to either the intervention group (red cell volume guideline transfusion group (RCV_{Grp})) or control group (haemoglobin threshold transfusion group (Hb_{Grp}))*. The former were transfused based both upon [Hb] and an estimate of RCV derived from body weight while Hb_{Grp} received blood products as per standard unit [Hb] based protocol (tables 4.1, 9.2 and 9.1). It should be noted that the algorithm provided for RCV_{Grp} was only used for the first 48h post-operatively as this corresponds with the phase of maximum haemodilution[177;185]. Standard unit protocol was applied for the period of operative management while clinical symptomatology and consultant preference determined whether patients were transfused RBCs after 48h post-operatively (table 4.1).

*Randomisation was carried out by randomly generated list (`www.randomization.com`) with order of recruitment determining the group allocated.

Table (9.1) *Intervention group RBC transfusion protocol for males. RBC = red blood cell and Hb = haemoglobin concentration.*

Body Weight (kg)	Hb Transfusion Threshold (g/dl)
≤ 77	**8.4**
78-97	**7.8**
≥ 98	**7.2**

Table (9.2) *Intervention group RBC transfusion protocol for females. RBC = red blood cell and Hb = haemoglobin concentration.*

Body Weight (kg)	Hb Transfusion Threshold (g/dl)
≤ 54	**8.5**
55-74	**8.0**
≥ 75	**7.5**

9.4.3 The Derivation of an Red Cell Volume Based Transfusion Guideline

As gender and weight were identified as major determinants of RCV in chapter 4 (page - 53), our previous data obtained in chapter 5 (page - 66) was divided into male and female categories with three further weight based sub-divisions[171;173;177]. The mean pre-operative RCV for each weight based subdivision, calculated as recommended by the ICSH, was multiplied by a threshold ratio allowing a theoretical RCV transfusion threshold to be calculated[171]. Finally, a dilution factor for both males and females, again from our previous work, was applied to give relevance to post-operative [Hb] (tables 9.2 and 9.1)[177]. Further details of how this algorithm was calculated can be found in appendix C (page - 185).

Although restrictive transfusion protocols have demonstrated the safety of [Hb] transfusion thresholds as low as 8g/dl in cardiac surgery patients, the protocol included several exclusion criteria to minimise risk[114];

- Drain losses greater than 500ml in the first post-operative hour*.
- Continuing losses greater than 250ml an hour for subsequent hours.
- Persistent or worsening metabolic acidosis (H+ $\geq$ 55 with standard base excess (SBE) $\geq$ - 6).
- Mean arterial pressure consistently $\leq$ 60mmHg
- ECG ST segment change suggestive of myocardial ischaemia.

An example of the algorithm provided for RCV_{Grp} (male 78-97kg) is given in figure 9.1.

9.4.4 Study Variables

9.4.4.1 Red Cell Transfusion

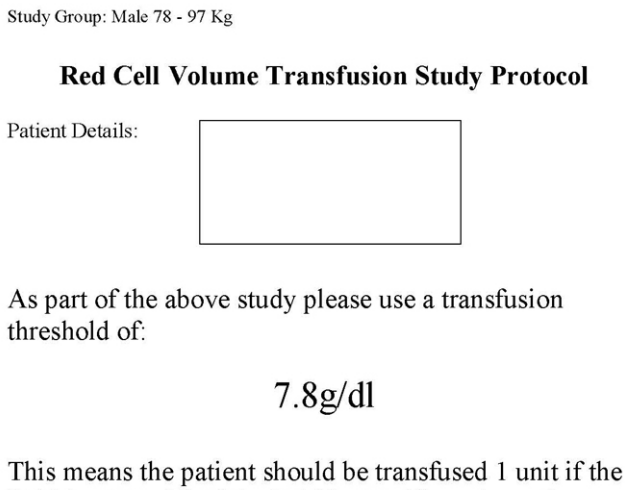
RBC transfusion was recorded in terms of; % patients transfused, average number of units per patient transfused (UPPT) and average number of units per patient operated (UPPO). Data was collected for both the initial 48h post-operatively, as governed by the RCV guideline in RCV_{Grp}, and total hospital stay.

9.4.4.2 Clinical Outcomes

The following variables were recorded to assess clinical patient outcome; inotrope requirement, ventilation time, cardiac arrythmia including atrial fibrillation, myocardial infarction (troponin $\geq$0.5 ng/ml), cerebro-vascular accident (CVA), chest infection and/or wound infection as confirmed by positive culture, discharge [Hb], maximum creatinine, ICU stay and overall length of hospital stay.

*As per unit policy to allow for the drainage of thoracic washout.

Figure (9.1) *Example study algorithm.*



Study Group: Male 78 - 97 Kg

Red Cell Volume Transfusion Study Protocol

Patient Details:

As part of the above study please use a transfusion threshold of:

7.8g/dl

This means the patient should be transfused 1 unit if the [Hb] is < 7.8g/dl. If the [Hb] is 7.8g/dl or above they should not be transfused.

No more than 1 unit should be given without rechecking [Hb].

Transfusion should be given on the basis of one [Hb] result below the transfusion threshold.

Protocol to be discontinued if;

- Drain losses greater than 500mls in the first post-operative hour
- Continuing losses greater than 250mls an hour for subsequent hours
- Persistent or worsening metabolic acidosis ($H^+ \geq 55$ with $BE \geq -6$)
- Mean arterial pressure consistently ≤ 60mmHg
- ECG ST segment change suggestive of myocardial ischaemia

9.4.4.3 Quality of life

In order to provide a baseline and post-operative assessment (at 3 months) of patient quality of life (QOL) the Euro QOL EQ-5D questionnaire was selected. The EQ-5D descriptive system consists of 5 dimensions of health (mobility, self-care, usual activities, pain/discomfort, anxiety/depression). Each dimension comprises three levels (no problems, some/moderate problems/extreme problems). An EQ-5D health state is defined by combining 1 level from each of the 5 dimensions. A further section (EQ-VAS) records patients self rated health status on a graduated (0-100) visual analogue scale[186;187].

9.4.5 Statistical Analysis

It was calculated from our previous research that a sample size of 86 patients would have 80% power to detect a difference of 0.7u in UPPO for the initial 48h post-operatively[177]. All variables were presented as mean (SD) unless non-normally distributed in which case median (SEM) is given. All linear outcome variables in the two groups were compared using the two group t-test. If the assumptions of the two group t-test were not met for any of these variables then logarithmic transformations or non-parametric tests were used as appropriate. Binary outcome variables were compared using Pearson's Chi Square test. A $P_{Val} \leq 0.05$ was considered statistically significant. SPSS version 13 was used for all statistical analysis.

Table (9.3) *Patient characteristics and lung function. RCV = red cell volume, Hb = haemoglobin concentration, P_{Val} = P value and BSA = body surface area. All data is presented as mean (SD) unless otherwise indicated.*

	RCV_{Grp} (n = 43)	**Hb_{Grp} (n = 43)**	P_{Val}
Patient Variables			
Age (y)	64.6 (10.6)	65.5 (10.0)	0.69
Gender (m/f)	31/12	29/14	0.64
Height (m)	167 (7.5)	168 (10.2)	0.42
Weight (kg)	78.4 (13.8)	83.3 (15.3)	0.12
BSA (m^2)	1.92 (0.19)	1.99 (0.23)	0.13
RCV (ml)*	1945 (50)	2183 (62)	0.14
Anti-platelet <7d (y/n)	19/24	22/21	0.52
Pre-op Creatinine (μmol/l)	99.5 (17.6)	98.4 (18.5)	0.77
Parsonnet Score	10.1 (7.3)	11.3 (6.9)	0.45
Euro Score	3.8 (2.7)	4.4 (2.5)	0.36
Lung Function			
Smoking Status (Current/Ex/Non)	5/27/11	7/20/16	0.32
FEV_1 (L/s)	2.6 (0.8)	2.6 (0.8)	0.80
FEV_1 % Predicted	96.8 (17.8)	93.4 (15.8)	0.40
FVC (L)	3.4 (0.9)	3.3 (1.0)	0.61
FVC % Predicted	100.1 (12.4)	96.3 (13.6)	0.13

* As the assumptions of the two group t-test were not met for this variable non-parametric analysis was used with data presented as median (SEM).

Table (9.4) *Pre-operative haematology. RCV = red cell volume, Hb = haemoglobin concentration, P_{Val} = P value, Hct = haematocrit, PTr = prothrombin time ratio, APTTr = activated partial thromboplastin time ratio and Plt = platelet count. All data is presented as mean (SD).*

	RCV_{Grp} (n = 43)	**Hb_{Grp} (n = 43)**	P_{Val}
Hb (g/dl)	13.6 (1.5)	13.7 (1.5)	0.75
Hct (%)	40.5 (4.0)	40.7 (3.8)	0.77
PTr	0.7 (0.1)	0.7 (0.1)	0.58
APTTr	0.9 (0.1)	0.9 (0.1)	0.91
Plt (x10^3/ml)	230 (68)	240 (61)	0.47
Fibrinogen (mg/dl)	3.2 (0.7)	3.3 (0.5)	0.25

9.5 Results

9.5.1 Demographic and Peri-operative Data

Ninety-seven adult elective cardiac surgery patients were recruited to the study over an eleven month period. Overall, eleven patients were subsequently excluded. Of these, in RCV_{Grp} four were subject to protocol error, two bled excessively (as defined in section 9.4.3), and one patient sustained an operative MI. Similarly, in Hb_{Grp} two protocol errors were observed with excessive blood loss in one patient and one operative MI.

Of the eighty-six patients completing the study, 46 underwent CABG (RCV_{Grp} = 27, Hb_{Grp} = 19), 20 a AVR (RCV_{Grp} = 9, Hb_{Grp} = 11), 1 a MVR (RCV_{Grp} = 1), 2 a AVR and MVR (Hb_{Grp} = 2), 15 a CABG and AVR (RCV_{Grp} = 5, Hb_{Grp} = 10) and 2 a CABG and MVR (RCV_{Grp} = 1, Hb_{Grp} = 1). No significant difference was observed between the groups for operation performed (P_{Val} = 0.28) or the surgeon performing the operation (P_{Val} = 0.07).

Table (9.5) *Operative characteristics. RCV = red cell volume, Hb = haemoglobin concentration, P_{Val} = P value, CPB = cardio-pulmonary bypass and ACC = aortic cross clamp. All data is presented as mean (SD) unless otherwise indicated.*

	$\mathbf{RCV}_{Grp}$ **(n = 43)**	$\mathbf{Hb}_{Grp}$ **(n = 43)**	$\mathbf{P}_{Val}$
Heparin (IU/kg)	471 (90)	483 (82)	0.53
Protamine (mg/kg)	3.0 (0.8)	3.0 (1.3)	0.93
CPB Time (min)	107 (57)	108 (34)	0.88
ACC Time (min)	69 (35)	70 (27)	0.93
Min Temp (^{0}C)	31.2 (1.7)	31.2 (1.9)	0.93
Max Temp (^{0}C)	36.4 (0.8)	36.4 (0.6)	0.73
Dilution Factor*	0.04 (0.03)	0.02 (0.02)	0.57
Aprotinin (y/n)	32/11	26/17	0.17

* As the assumptions of the two group t-test were not met for this variable non-parametric analysis was used with data presented as median (SEM).

Pre-operative patient characteristics were comparable for both groups in terms of; age (RCV_{Grp} = 64.6y SD 10.6, Hb_{Grp} = 65.5y SD 10.0, P_{Val} = 0.69), gender (m/f, RCV_{Grp} = 31/12, Hb_{Grp} =29/14, P_{Val} = 0.64), BSA (RCV_{Grp} = 1.92m^2 SD 0.19, Hb_{Grp} = 1.99m^2 SD 0.23, P_{Val} = 0.13), RCV* (median, RCV_{Grp} = 1945ml SEM 50, Hb_{Grp} = 2183ml SEM 62, P_{Val} = 0.14), anti-platelet therapy within 7 days of operation (y/n, RCV_{Grp} = 19/24, Hb_{Grp} = 22/21, P_{Val} = 0.52), creatinine (RCV_{Grp} = 99.5μmol/l SD 17.6, Hb_{Grp} = 98.4μmol/l SD 18.5, P_{Val} = 0.77) and Euro score (RCV_{Grp} = 3.8 SD 2.7, Hb_{Grp} = 4.4 SD 2.5, P_{Val} = 0.36). A full summary of all the pre-operative patient characteristics examined is given in table 9.3.

As ventilation time/chest infection were recorded outcome measures, smoking status and pulmonary function tests were documented for all patients. Again these were comparable pre-operatively between the two groups (table 9.3).

*Calculated as recommended by the ICSH[171]

No significant difference was noted pre-operatively for the haematological variables of; [Hb] (RCV_{Grp} = 13.6g/dl SD 1.5, Hb_{Grp} = 13.7g/dl SD 1.5, P_{Val} = 0.75), Hct (RCV_{Grp} = 40.5% SD 4.0, Hb_{Grp} = 40.7% SD 3.8, P_{Val} = 0.77), prothrombin time ratio (PTr) (RCV_{Grp} = 0.7 SD 0.1, Hb_{Grp} = 0.7 SD 0.1, P_{Val} = 0.58), activated partial thromboplastin time ratio (APTTr) (RCV_{Grp} = 0.9 SD 0.1, Hb_{Grp} = 0.9 SD 0.1, P_{Val} = 0.91), Plt (RCV_{Grp} = 230x10^3/ml SD 68, Hb_{Grp} = 240x10^3/ml SD 61, P_{Val} = 0.47) and fibrinogen (RCV_{Grp} = 3.2mg/dl SD 0.7, Hb_{Grp} = 3.3mg/dl SD 0.5, P_{Val} = 0.25) (table 9.4)

Similarly, operative data was comparable for; heparin dose (RCV_{Grp} = 471IU/kg SD 90, Hb_{Grp} = 483IU/kg SD 82, P_{Val} = 0.53), protamine dose (RCV_{Grp} = 3.0mg/kg SD 0.8, Hb_{Grp} = 3.0mg/kg SD 1.3, P_{Val} = 0.93), CPB time (RCV_{Grp} = 107min SD 57, Hb_{Grp} = 108min SD 34, P_{Val} = 0.88), ACC time (RCV_{Grp} = 69min SD 35, Hb_{Grp} = 70min SD 27, P_{Val} = 0.93), minimum CPB temperature (RCV_{Grp} = 31.2^{0}C SD 1.7, Hb_{Grp} = 31.2^{0}C SD 1.9, P_{Val} = 0.93), max re-warm temperature (RCV_{Grp} = 36.4^{0}C SD 0.8, Hb_{Grp} = 36.4^{0}C SD 0.6, P_{Val} = 0.73), dilution factor (CPB clear fluid volume/BV) (median, RCV_{Grp} = 0.04 SD 0.03, Hb_{Grp} = 0.02 SD 0.02, P_{Val} = 0.57) and Aprotinin administration (y/n, RCV_{Grp} = 32/11, Hb_{Grp} = 26/17, P_{Val} = 0.17) (table 9.5). Post-operative drain loss was also comparable between the two groups (RCV_{Grp} = 897ml SD 542, Hb_{Grp} = 881ml SD 446, P_{Val} = 0.89).

9.5.2 Red Cell Transfusion

Overall, a significantly smaller percentage of RCV_{Grp} patients were transfused (RCV_{Grp} = 32.6%, Hb_{Grp} = 53.5%, P_{Val} = 0.05). For the first 48h following ICU_{Ret}, RCV_{Grp} were transfused fewer RBCs in terms of UPPO (RCV_{Grp} = 0.42u, Hb_{Grp} = 0.81, P_{Val} = 0.04) but not UPPT (RCV_{Grp} = 1.29u, Hb_{Grp} = 1.59, P_{Val} = 0.17). Similar findings were noted for total RBCs transfused with UPPO (RCV_{Grp} = 0.60u, Hb_{Grp} = 1.19, P_{Val} = 0.03) and UPPT (RCV_{Grp} = 1.86u, Hb_{Grp} = 2.22, P_{Val} = 0.38). Insufficient numbers of other blood components were transfused to allow meaningful statistical analysis.

9.5.3 Outcome Measures

For both groups, several of the outcome measures occurred to infrequently to allow meaningful analysis. In RCV_{Grp}, 1 patient developed a deep wound infection. In Hb_{Grp}, 1 patient developed a deep wound infection, 2 required renal replacement therapy and 1 suffered a CVA. No patients died or met any of the post-operative exclusion criteria with the exception of drain loss.

Of those examined statistically, total hospital stay was greater in Hb_{Grp} (median, RCV_{Grp} = 5.9d SEM 0.8, Hb_{Grp} = 6.8d SEM 2.2, P_{Val} = 0.02) while RCV_{Grp} had a higher inotrope requirement (RCV_{Grp} = 25, Hb_{Grp} = 15, P_{Val} = 0.03). No significant difference was identified for the remaining variables of; ventilation time (median, RCV_{Grp} = 15.3h SEM 1.9, Hb_{Grp} = 12.7h SEM 6.3, P_{Val} = 0.14), ICU stay (median, RCV_{Grp} = 1.0d SEM 0.1, Hb_{Grp} = 1.0d SEM 0.4, P_{Val} = 0.67), discharge [Hb] (RCV_{Grp} = 10.2g/dl SD 1.0, Hb_{Grp} = 10.2g/dl SD 1.1, P_{Val} = 0.90), maximum creatinine (RCV_{Grp} = 103μmol/l SD 24, 116μmol/l SD 38, P_{Val} = 0.07), arrythmia (RCV_{Grp} = 10, Hb_{Grp} = 11, P_{Val} = 0.80), pneumonia (RCV_{Grp} = 12, Hb_{Grp} = 11, P_{Val} = 0.81) and superficial wound infection (RCV_{Grp} = 2, Hb_{Grp} = 3, P_{Val} = 0.65) (table 9.6).

Table (9.6) *Outcome measures. RCV = red cell volume, Hb = haemoglobin concentration, P_{Val} = P value, ICU = intensive care unit and HDU = high dependency unit. All data is presented as mean (SD) unless otherwise indicated.*

	$\mathbf{RCV}_{Grp}$ **(n = 43)**	$\mathbf{Hb}_{Grp}$ **(n = 43)**	$\mathbf{P}_{Val}$
Ventilation Time (h)*	15.3 (1.9)	12.7 (6.3)	0.14
ICU Stay (d) *	1.0 (0.1)	1.0 (0.4)	0.67
Hospital Stay (d) *	5.9 (0.8)	6.8 (2.2)	0.02
Discharge Hb (g/dl)	10.2 (1.0)	10.2 (1.1)	0.90
Max Creatinine (μmol/l)	103 (24)	116 (38)	0.07
Inotropes (n)	25	15	0.03
Arrythmia (n)	10	11	0.80
Pneumonia (n)	12	11	0.81
Superficial Wound Infection (n)	2	3	0.65

* As the assumptions of the two group t-test were not met for this variable non-parametric analysis was used with data presented as median (SEM).

9.5.4 Quality of Life

As discussed in section 9.4.4.3, QOL was assessed both pre-operatively and at 3 months following surgery. Pre-operatively, of the EQ-5D descriptive parameters no significant difference was noted in terms of % patients with any problems for; mobility (RCV_{Grp} = 44.2%, Hb_{Grp} = 62.8%, P_{Val} = 0.08), self care (RCV_{Grp} = 4.7%, Hb_{Grp} = 11.6%, P_{Val} = 0.24), usual activity (RCV_{Grp} = 55.8%, Hb_{Grp} = 67.4%, P_{Val} = 0.27), pain (RCV_{Grp} = 60.5%, Hb_{Grp} = 53.5%, P_{Val} = 0.51) and anxiety (RCV_{Grp} = 32.6%, Hb_{Grp} = 41.9%, P_{Val} = 0.37). Similarly, the EQ-VAS measured health assessment score (0-100) was found to be comparable between the two groups (RCV_{Grp} = 61.2 SD 17.6, Hb_{Grp} = 60.4 SD 14.3, P_{Val} = 0.83).

In RCV_{Grp}, thirty-nine patients (n = 39/43) returned completed questionnaires while the corresponding figure in Hb_{Grp} was thirty-six (n = 36/43). Of the EQ-5D questions studied at 3 months, no significant difference was noted in terms of % patients with any problems for; mobility (RCV_{Grp} = 30.7%, Hb_{Grp} = 22.2%, P_{Val} = 0.44), self care (RCV_{Grp} = 7.7%, Hb_{Grp} = 11.1%, P_{Val} = 0.55), usual activity (RCV_{Grp} = 53.8%, Hb_{Grp} = 44.4%, P_{Val} = 0.45), pain (RCV_{Grp} = 53.9%, Hb_{Grp} = 63.9%, P_{Val} = 0.42) and anxiety (RCV_{Grp} = 28.2%, Hb_{Grp} = 27.7%, P_{Val} = 0.6). Similarly, the EQ-VAS measured health assessment score (0-100) was found to be comparable between the two groups at 3 months (RCV_{Grp} = 74.3 SD 16.2, Hb_{Grp} = 77.3 SD 12.3, P_{Val} = 0.36).

Table (9.7) *Pre-operative quality of life. RCV = red cell volume, Hb = haemoglobin concentration and P_{Val} = P value. All data is presented as % patients with any difficulty apart from health state which is represented as mean 0-100 self assessment score (SD).*

	$\mathbf{RCV}_{Grp}$ **(n = 43)**	$\mathbf{Hb}_{Grp}$ **(n = 43)**	$\mathbf{P}_{Val}$
Mobility	44.2	62.8	0.08
Self Care	4.7	11.6	0.24
Usual Activity	55.8	67.4	0.27
Pain	60.5	53.5	0.51
Anxiety	32.6	41.9	0.37
Health State	61.2 (17.6)	60.4 (14.3)	0.83

Table (9.8) *Post-operative quality of life. RCV = red cell volume, Hb = haemoglobin concentration and P_{Val} = P value. All data is presented as % patients with any difficulty apart from health state which is represented as mean 0-100 self assessment score (SD).*

	$\mathbf{RCV}_{Grp}$ **(n = 39/43)**	$\mathbf{Hb}_{Grp}$ **(n = 36/43)**	$\mathbf{P}_{Val}$
Mobility	30.7	22.2	0.44
Self Care	7.7	11.1	0.55
Usual Activity	53.8	44.4	0.45
Pain	53.9	63.9	0.42
Anxiety	28.2	27.7	0.63
Health State	74.3 (16.2)	77.3 (12.6)	0.36

9.6 Summary of Principle Findings

Demographic and peri-operative data was comparable for both groups. Patients in Hb_{Grp} received significantly more RBCs in terms of % patients transfused and UPPO, but not UPPT. Of the outcome measures examined; deep wound infection, renal impairment and CVA were of insufficient occurrence to allow meaningful comparison. Significant associations were found with Hb_{Grp} exhibiting a longer period of hospital stay while a greater number of RCV_{Grp} patients received inotropic therapy. The remaining statistically examined variables of; ventilation time, ICU stay, discharge [Hb], maximum creatinine, arrythmia, pneumonia and superficial wound infection were non-significant between the two groups. In addition, no significant difference was noted in response to any of the QOL questions at 3 months.

Please see section 12.7 (page - 163) for a full discussion of the above findings.

Chapter 10

Lung Injury Following Red Cell Transfusion: A Pilot Investigation

10.1 Background

As discussed in section 2.2 (page - 36), varying degrees of ALI are common following cardiac surgery[77;149]. Although RBC transfusion has been described in relation to the more extreme forms of ALI as TRALI, the subtler forms of the condition are less well documented and almost certainly under recognised[74]. Diagnosis is often difficult as the various measurable inflammatory mediators may be non-specific for the causative pulmonary endothelial injury. In relation to the use of CPB, several authors have attempted to document the association between exhaled NO analysis and ALI. Reportedly, this technique has the advantage that any reduction in exhaled NO is directly proportional to the degree of pulmonary endothelial damage[152–154].

This study was designed to explore whether RBC transfusion could be associated with subtler forms of ALI, as measured by exhaled NO analysis, following cardiac surgery.

10.2 Aim

To determine the association between pulmonary endothelial injury and RBC transfusion.

10.3 Materials and Methods

10.3.1 Patients

Please see section 9.4 (108)*.

10.3.2 Patient Management

Please see section 4.3.2 (page - 56).

10.3.3 Red Cell Transfusion

Prior to surgery patients were randomised to either the RCV_{Grp} or the Hb_{Grp}. The former were transfused as per the RCV guideline described in appendix C (page - 185) while group Hb_{Grp} received blood products as per the standard unit protocol previously described in section 4.3.3 (page - 56).

10.3.4 Study Variables

10.3.4.1 Nitric Oxide Analysis

In order to quantify the degree of peri-operative lung injury NO measurements were taken. Previous research has demonstrated that reduction in NO is associated with the severity of the pulmonary endothelial damage sustained[152–154]. The adherence of activated neutrophils to pulmonary endothelium with resultant endothelial damage is widely recognised as the key component of TRALI[74].

*The cohort for this study was taken from patients recruited during the latter three months of the study described in Chapter 9 (page - 106). As such, this section is as previously described.

Figure (10.1) *Nioxmino nitric oxide analyser. Reproduced from Aerocrine Inc (http://www.aerocrine.com).*

Exhaled NO analysis was performed at the time of hospital admission, and at 48h post-operatively. NO analysis was performed by the hand held nioxmino device (figure 10.1)*. Patients are asked to make a 10s exhalation through the device at a pressure of 10 - 20cm H_2O maintaining a fixed flow rate of 50ml/s. As exhalation is against positive pressure, the effects of nasal nitrate contamination are minimised by the apposition of the soft palate against the posterior pharyngeal wall. Results are provided on a digital read out expressed as NO parts per billion (ppb)[152–154] †. The primary outcome variable considered was % change in NO (ΔNO %) between the two study time points. Although this technique has been validated for use in asthmatics, this represents a

*Aerocrine AB, Solna, Sweden.

†Although this technique has been validated for use in asthmatics, this represents a new and novel application.

10.3.4.2 Other Variables

All demographic and peri-operative data was recorded. In addition, pulmonary function tests (PFTs) for forced expiratory volume 1s (FEV1) and forced vital capacity (FVC) were performed prior to operation. Where documented, RCV was calculated as recommended by the ICSH (equations A.2 and A.3)[171]. BSA was determined as recommended by Gehan and George (equation A.1)[170].

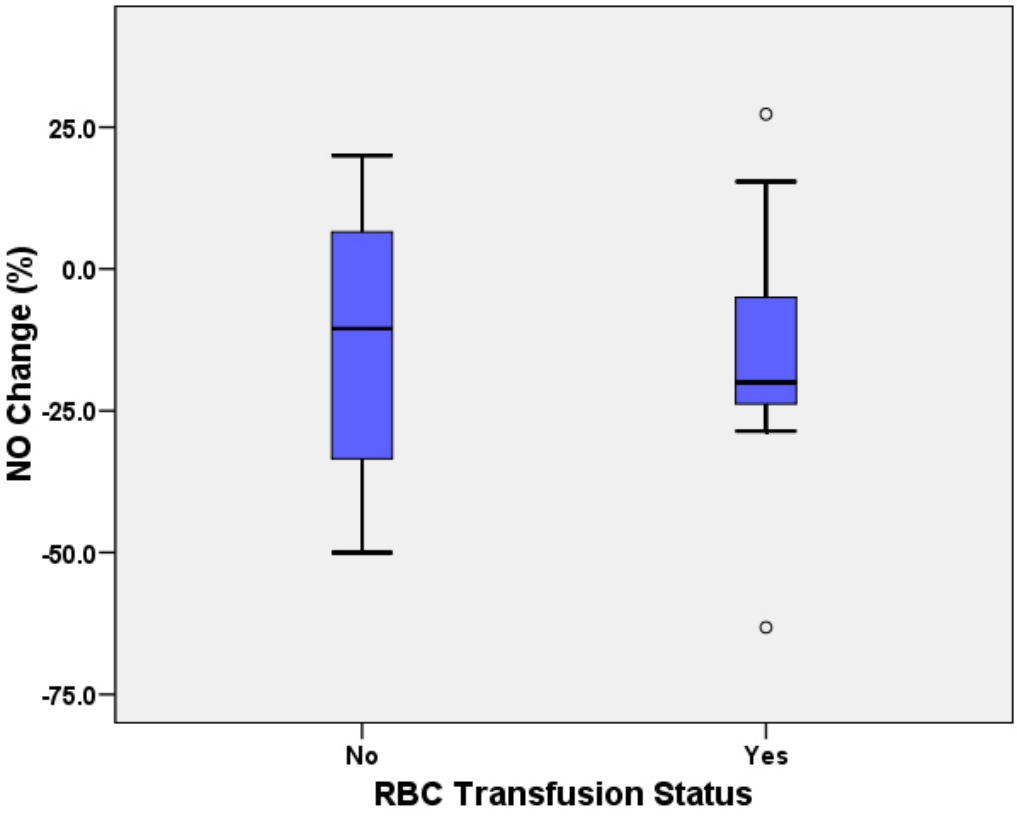
10.3.5 Statistical Analysis

As this was an exploratory study, using patients from an ongoing study cohort, no formal power calculation was performed. All linear outcome variables in the two groups were compared using the two group t-test. If the assumptions of the two group t-test were not met for any of these variables then transformations of these variables or non-parametric tests were used as appropriate. Binary outcome variables were compared using Fisher's exact test. A P_{Val} of ≤ 0.05 was considered statistically significant. SPSS version 13 was used for all statistical analysis.

10.4 Results

Twenty-four adult elective cardiac surgery patients were recruited to the study over a three month period. Eight patients were subsequently excluded due to an inability to complete the exhaled NO breath test at 48h post-operatively. On analysing the data, another difficulty was identified. Namely, that no significant difference existed in RBC transfusion status between the two groups (y/n, $RCV_{Grp} = 5/1$, $Hb_{Grp} = 4/5$, $P_{Val} = 0.36$). This prompted a re-appraisal of the way the results were analysed. It was decided that although any results would be limited, the best course of action was to analyse patients per transfusion status (red blood cell transfusion group (RBC_{Grp}) vs no red blood cell transfusion group ($NoRBC_{Grp}$)). In addition, all study variables were correlated (r) with ΔNO %.

Figure (10.2) *Boxplots for percentage change in NO vs transfusion status. NO = nitric oxide.*



10.4.1 Red Cell Transfusion Status

Of the sixteen patients completing the study, 10 underwent CABG ($\text{RBC}_{Grp} = 6$, $\text{NoRBC}_{Grp} = 4$), 5 received a AVR ($\text{RBC}_{Grp} = 3$, $\text{NoRBC}_{Grp} = 2$) and 1 a CABG and AVR ($\text{NoRBC}_{Grp} = 1$). No significant difference was observed between the groups for operation performed ($\text{P}_{Val} = 0.50$) or the surgeon performing the operation ($\text{P}_{Val} = 0.30$).

When considering the individual patient variables, no significant difference was observed for; age (RBC_{Grp} = 68y SEM 2.1, $NoRBC_{Grp}$ = 57y SEM 1.9, P_{Val} = 0.61), gender (m/f, RBC_{Grp} = 7/2, $NoRBC_{Grp}$ = 7/0, P_{Val} = 0.48), height (RBC_{Grp} = 171m SEM 2.9, $NoRBC_{Grp}$ = 179m SEM 2.0, P_{Val} = 0.84), weight (RBC_{Grp} = 76.9kg SEM 4.1, $NoRBC_{Grp}$ = 102.2kg SEM 7.6, P_{Val} = 0.25), BSA (RBC_{Grp} = 1.9m^2 SEM 0.1, $NoRBC_{Grp}$ = 2.3m^2 SEM 0.1, P_{Val} = 0.30), initial RCV (RBC_{Grp} = 1961ml SEM 110, $NoRBC_{Grp}$ = 2550ml SEM 660, P_{Val} = 0.68), pre-operative [Hb] (RBC_{Grp} = 12.9g/dl SEM 0.3, $NoRBC_{Grp}$ = 14.5g/dl SEM 0.5, P_{Val} = 0.06), euro score (RBC_{Grp} = 4.7 SEM 0.7, $NoRBC_{Grp}$ = 2.0 SEM 0.5, P_{Val} = 0.61), smoking (e/c/n, RBC_{Grp} = 5/0/4, $NoRBC_{Grp}$ = 2/1/4, P_{Val} 0.36), FEV1 (RBC_{Grp} = 2.6l/s SEM 0.2, $NoRBC_{Grp}$ = 3.3l/s SEM 0.2, P_{Val} 0.47) and FVC (RBC_{Grp} = 3.6l SEM 0.3, $NoRBC_{Grp}$ = 4.4l SEM 0.2, P_{Val} = 0.68) 10.1.

Similarly, operative data was comparable for; CPB time (RBC_{Grp} = 113min SEM 13.8, $NoRBC_{Grp}$ = 108min SEM 11.2, P_{Val} = 0.54), ACC time (RBC_{Grp} = 68min SEM 12.9, $NoRBC_{Grp}$ = 68min SEM 10.6, P_{Val} = 0.47), minimum CPB temperature (RBC_{Grp} = 31.7^{0}C SEM 0.8, $NoRBC_{Grp}$ = 31.1^{0}C SEM 0.9, P_{Val} = 0.30), max re-warm temperature (RBC_{Grp} = 36.6^{0}C SEM 0.4, $NoRBC_{Grp}$ = 36.3^{0}C SEM 0.3, P_{Val} = 0.61) and ventilation time (RBC_{Grp} = 13.7h SEM 2.0, $NoRBC_{Grp}$ = 7.7h SEM 2.2, P_{Val} = 0.02) (table 10.1).

For the primary outcome variable of ΔNO %, RBC transfusion status was not found to have statistically significant association (RBC_{Grp} = 14.7% SEM 8.8, $NoRBC_{Grp}$ = 13.5% SEM 10.4, P_{Val} = 0.30) (figure 10.2).

10.4.2 Correlation Coefficient for Study Variables

The continuous patient variables of; age (r = 0.15, P_{Val} = 0.59), height (r = 0.18, P_{Val} = 0.50), weight (r = -0.13, P_{Val} = 0.63), BSA (r = -0.09, P_{Val} = 0.75), initial patient RCV (r = -0.06, P_{Val} = 0.84), pre-operative [Hb] (r = -0.36, P_{Val} = 0.17), euro score (r = 0.06, P_{Val} = 0.84), FEV1 (r = -0.13, P_{Val} = 0.65) and FVC (r = -0.05, P_{Val} = 0.86) demonstrated no significant correlation with ΔNO%.

Table (10.1) *Study variables vs transfusion status. P_{Val} = P value, BSA = body surface area, RCV = red cell volume, Hb = haemoglobin, FEV1 = forced expiratory volume 1s, FVC = forced vital capacity, CPB = cardio-pulmonary bypass, ACC = aortic cross clamp and NO = nitric oxide. All data is presented as mean (SEM).*

	Transfusion Status		
Variable	**Yes (n = 9)**	**No (n = 7)**	**P_{Val}**
Age (y)	68 (2.1)	57 (1.9)	0.61
Gender (m/f)	7/2	7/0	0.48
Height (m)	171 (2.9)	179 (2.0)	0.84
Weight (kg)	76.9 (4.1)	102.2 (7.6)	0.25
BSA (m^2)	1.9 (0.1)	2.3 (0.1)	0.30
Initial RCV (ml)	1961 (110)	2550 (660)	0.68
Pre-op Hb (g/dl)	12.9 (0.3)	14.5 (0.5)	0.06
Euro Score	4.7 (0.7)	2.0 (0.5)	0.61
Smoking (ex/current/non)	5/0/4	2/1/4	0.36
FEV1 (l/s)	2.6 (0.2)	3.3 (0.2)	0.47
FVC (l)	3.6 (0.3)	4.4 (0.2)	0.68
CPB Time (min)	113 (13.8)	108 (11.2)	0.54
ACC Time (min)	68 (12.9)	68 (10.6)	0.47
Min Temp (^{0}C)	31.7 (0.8)	31.1 (0.9)	0.30
Max Temp (^{0}C)	36.6 (0.4)	36.3 (0.3)	0.61
Ventilation Time (h)	13.7 (2.0)	7.7 (2.2)	0.02
NO Reduction (%)	14.7 (8.8)	13.5 (10.4)	0.30

Findings were similar for the peri-operative parameters of; CPB time (r = -0.22, P_{Val} = 0.42), ACC time (r = -0.10, P_{Val} = 0.73), minimum CPB temperature (r = 0.15, P_{Val} = 0.58), maximal rewarm temperature (r = 0.04, P_{Val} = 0.87), ventilation time (r = -0.09, P_{Val} = 0.75) and units RBCs transfused (r = -0.22, P_{Val} = 0.42).

Table (10.2) *Correlation co-efficient (r) for study variables vs percentage NO reduction. NO = nitric oxide, P_{Val} = P value, BSA = body surface area, RCV = red cell volume, Hb = haemoglobin, FEV1 = forced expiratory volume 1s, FVC = forced vital capacity, CPB = cardio-pulmonary bypass, ACC = aortic cross clamp and RBC = red blood cell.*

Variable	**Correlation Co-efficient (r)**	**P_{Val}**
Age (y)	0.15	0.59
Height (m)	0.18	0.50
Weight (kg)	-0.13	0.63
BSA (m^2)	-0.09	0.75
Initial RCV (ml)	-0.06	0.84
Pre-op Hb (g/dl)	-0.36	0.17
Euro Score	0.06	0.84
FEV1 (l/s)	-0.13	0.65
FVC (l)	-0.05	0.86
CPB Time (min)	-0.22	0.42
ACC Time (min)	-0.10	0.73
Min Temp (^{0}C)	0.15	0.58
Max Temp (^{0}C)	0.04	0.87
Ventilation Time (h)	-0.09	0.75
RBC's (u)	-0.22	0.42

10.5 Summary of Principle Findings

Two principle difficulties were encountered during this study. Firstly, a large proportion of patients were unable to complete the exhaled NO analysis at 48h post-operatively (33%). Secondly, no significant difference in RBC transfusion was observed between RCV_{Grp} and Hb_{Grp}. Thus, the analysis was performed based upon RBC transfusion status and the calculation of correlation coefficients, limiting the value of the study.

When considering RBC transfusion status, all demographic and peri-operative data was broadly comparable between the two groups. Within the constraints of this small pilot study RBC transfusion status was not found to influence ΔNO %. Calculated correlation coefficients were all similarly non-significant when the various study parameters were examined versus ΔNO %.

Please see section 12.8 (page - 164) for a full discussion of the above findings.

Chapter 11

A Comparison of Individualised and Modified Activated Clotting Time Based Techniques for Anti-coagulation During Cardio-pulmonary Bypass

11.1 Background

Currently, heparin is considered the pharmacological anti-coagulant agent of choice for CPB. Usually, activated clotting time (ACT) is employed to assess the adequacy of heparinisation by virtue of its simplicity, safety, cost and relative effectiveness[158;160;161]. On the negative side however, ACT may be prolonged by both haemodilution and hypothermia, both of which are invariably encountered during CPB[162–164]. The resultant inadequate anti-coagulation, with exposure of blood to both foreign and intrinsic surfaces, may lead to coagulation activation and thrombin generation[165]. In order to avoid this potentially detrimental scenario, the use of HMSs has been advocated[164;166–169]. The objective of this study was to compare the effects of HMS* and modified ACT based anti-coagulation strategies on; haemostatic activation, post-operative blood loss and blood product requirement.

11.2 Aim

To evaluate the impact of both HMS and modified ACT based anti-coagulation management on; coagulation, thrombin generation, fibrinolysis, anti-thrombin III (AT-III), post-operative blood loss and blood product requirement.

*Hepcon, Medtronic Inc, Minneapolis, USA.

11.3 Materials and Methods

11.3.1 Patients

Ethical approval was gained from LREC. All adult (age $\geq$ 16y) elective cardiac surgery patients presenting for operation between January and September 2004 were considered for recruitment. Exclusion criteria were; the inability to provide informed consent, pre-operative anaemia ([Hb] $<$ 13g/dl for males and $<$ 11g/dl for females), anti-platelet therapy taken within seven days prior to surgery, long term warfarin therapy, pre-operative heparin, disordered coagulation, emergency surgery, redo surgery and surgical re-exploration.

On the day of operation, patients recruited to the study were randomised to receive either a standard dose of heparin (activated clotting time management group (ACT_{Grp})) or an individualised dose based upon the HMS (heparin management system group (HMS_{Grp}))*. In all instances the operating surgeon and staff responsible for post-operative care were blinded as to the randomisation.

11.3.2 Patient Management

Anaesthesia was induced and maintained with a combination of; fentanyl, midazolam, propofol, enflurane and pancuronium. Prior to the commencement of surgery standard monitoring was instituted (arterial pressure, central venous pressure, ECG, urinary catheter and nasal temperature). Anti-coagulation for CPB, and its reversal, was as per the allocated study group protocol, described in detail below (section 11.3.5). The extra-corporeal circuit consisted of a roller pump†, flexible venous reservoir, vent and cardiotomy suction‡. The circuit was primed with 2l Hartmann's solution, 50mmol bicarbonate, 150mmol mannitol and 8000IU of heparin for a total volume of 2250ml. When the individual procedure had been completed, patients were fully rewarmed to 37^0C and CPB discontinued. All residual pump blood was returned to the patient.

*Randomisation was carried out by randomly generated list (`www.randomization.com`) with order of recruitment determining the group allocated.

†Stockert Instruments, Munich, Germany.

‡Medtronic Inc, Minneapolis, USA.

Table (11.1) *Clotting factor administration protocol. Plt = platelet count, PTr = pro-thrombin time ratio, APTTr = activated partial thromboplastin time ratio and Fib = fibrinogen.*

Abnormality	**A**dminister
Plt < 100,000ml	Platelets
PTr > 1.6	Fresh Frozen Plasma
APTTr > 1.6	Fresh Frozen Plasma
Fib < 1g/L	Fresh Frozen Plasma

Thoracic drainage was achieved with a combination of pericardial, mediastinal and pleural drains where required. Crystalloid was given in the ICU at a rate of 0.5ml/kg/h with colloid administered (4.5% human albumin solution) where required for hypotension (MAP < 60mmHg) and/or poor urine output (< 0.5ml/kg/h).

11.3.3 Red Blood Cell Transfusion

Please see section 4.3.3 (page - 56).

11.3.4 Blood Component Administration

Blood components for both groups were administered as per local protocol (table 11.1). It should be noted factors were only given in the presence of significant post-operative blood loss ($\geq$ 500ml in the first post-operative hour and/or continuing losses $\geq$ 250ml for subsequent hours).

Figure (11.1) *Hepcon heparin management system (HMS).*

11.3.5 Anti-coagulation Management

11.3.5.1 ACT Group

In ACT_{Grp}, ACT was measured using a Celite/Kaolin/Glass activator* to minimise Aprotinin effect, on a Hemochron 401 analyser†. A baseline ACT measurement was performed on induction of anesthesia. An initial heparin bolus of 300IU/kg was given to the patient with a further 8000IU added to the extracorporeal circuit prime. During CPB, 4000IU of heparin was given for each litre of crystalloid or unit of RBCs infused. ACTs were checked post initial heparin bolus and at 10min and every 30min of CPB. Where the ACT was less than 480s a dose-response algorithm, as devised by Bull et al, was used to calculate the additional heparin dose required (equation D.1)[157;160].

*Max-ACT, Helena Labs, Sunderland, UK.
†International Technidyne Corp, Edison, USA.)

At the termination of CPB, protamine sulphate was given at a ratio of 1:1 of the, dose-response curve calculated, estimated residual circulating heparin volume (equation D.2)[157;160]. Further boluses were given, similarly calculated, following protamine administration and and on ICU_{Ret} where the ACT remained above the pre-operative target level*.

11.3.5.2 HMS Group

In HMS_{Grp}, HDR was performed by the HMS at the induction of anaesthesia. Heparin was subsequently given at the dose calculated to achieve a target ACT of greater than 480s, usually resulting in an empirical BH_{Conc} target of 4-5u/ml†. The dose of heparin contained within the pump priming solution was deducted from the bolus dose given to the patient. During CPB, the BH_{Conc} was measured at 10min and every 30min of CPB by HPT. Where BH_{Conc} fell below the desired target level additional heparin was given at the dose calculated by the HMS (figure 11.1).

Following the termination of CPB, HPT was again used to calculate the protamine dose (again at a ratio of 1:1) required to neutralise all residual circulating heparin. This was repeated post-protamine and on ICU_{Ret} with further protamine given as indicated †. Further detail on the principles behind the HMS can be found in appendices D.2 and D.3 (page - 190)

*No further protamine was given if ACT remained elevated beyond this point unless residual heparin effect was identified on formal coagulation screen

†Although heparin dosage is expressed as IU, BH_{Conc} and plasma heparin concentration (PH_{Conc}) is given in United States Pharmacopeia (USP) units (u). The correction factor for IU is 0.88. This was done to ensure accurate comparison with similar studies.

11.3.6 Study Variables

All blood samples, apart from the pre-operative screen, were collected from the radial artery catheter inserted prior to the induction of anaesthesia. Samples were collected at the following time points; induction of anaesthesia (defined for the reporting of results as t = 0), 5min post-heparin, 10min of CPB, every 30min of CPB, termination of CPB, 5min post-protamine and ICU_{Ret}.

Several routine and laboratory markers of coagulation were selected for the study analysis (table 11.2). Thrombin anti-thrombin (TAT) was selected to record the degree of thrombin generation while D-Dimer levels were used to quantify fibrinolysis*. AT-III assay was used to give an indication of both coagulation activation and heparin sensitivity†.

For both groups, PH_{Conc} was calculated from the, HPT measured, BH_{Conc} (equation D.3). This method has been shown to be accurate when compared to sensitive anti-Xa chromogenic substrate assays[163].

11.3.7 Statistical Analysis

It was calculated from previous similar research that a sample size of 36 patients would have 80% power to detect a difference of 19g/ml in thrombin generation (TAT assay)[169]. All variables were presented as mean (SEM). A Student's t-test was performed to determine the statistical significance of any observed difference between variables for consecutive time points and between the study groups. Where the assumptions of the two group t-test were not met non-parametric analysis was used. Binary variables were compared by use of Fishers exact test. A $P_{Val} \leq 0.05$ was considered statistically significant. SPSS version 13 was used for all statistical analysis.

*Both by ELISA, Dade Behring, Milton Keynes, UK

†Chromogenic Assay, Quadrateck, Epsom, UK

Table (11.2) *Study variables. ACT_{Grp} = activated clotting time group, HMS = heparin management system, ACT = activated clotting time, Hb = haemoglobin concentration, Hct = haematocrit, Plt = platelet count, HDR = heparin dose response PT = pro-thrombin time ratio, APTT = activated partial thromboplastin time, Fib = fibrinogen, TAT = thrombin anti-thrombin, AT-III = anti-thrombin III, HPT = heparin protamine titration and ICU = intensive care unit.*

	ACT$_{Grp}$	**H**MS$_{Grp}$
Induction	ACT, Hb, Hct, Plt PT, APTT, Fib	HDR, ACT, Hb, Hct Plt, PT, APTT, Fib
Post-heparin	TAT, D-Dimer, AT-III Fib, ACT, HPT, Hb, Hct	TAT, D-Dimer, AT-III Fib, ACT, HPT, Hb, Hct
10min CPB	ACT, HPT, Hb, Hct	ACT, HPT, Hb, Hct
Every 30min CPB	ACT, HPT, Hb, Hct	ACT, HPT, Hb, Hct
Termination CPB	TAT, D-Dimer, AT-III Fib, ACT, HPT, Hb, Hct	TAT, D-Dimer, AT-III Fib, ACT, HPT, Hb, Hct
Post-protamine	HPT, ACT, Hb, Hct Plt, PT, APTT, Fib	HPT, ACT, Hb, Hct Plat, PT, APTT, Fib
Return ICU	HPT, ACT, Hb, Hct Plt, PT, APTT, Fib	HPT, ACT, Hb, Hct Plat, PT, APTT, Fib

11.4 Results

11.4.1 Demographic and Peri-operative Data

Forty-one adult elective cardiac surgery patients were recruited to the study over a nine month period. Two patients in ACT_{Grp} (n = 2/20) and one in HMS_{Grp} (n = 1/21) were subsequently excluded due to re-operation for surgical bleeding. Of the thirty-eight patients completing the study, 21 underwent CABG (ACT_{Grp} = 12, HMS_{Grp} = 9), 15 a AVR (ACT_{Grp} = 7, HMS_{Grp} = 8) and 2 a ASD repair (ACT_{Grp} = 1, HMS_{Grp} = 1). No significant difference was observed between the groups for operation performed (P_{Val} = 0.94) or the surgeon performing the operation (P_{Val} = 0.40).

Pre-operative characteristics were comparable for both groups in terms of; age (ACT_{Grp} = 60.4y SEM 2.8, HMS_{Grp} = 64.8y SEM 2.3, P_{Val} = 0.24), weight (ACT_{Grp} = 83.5kg SEM 4.6, HMS_{Grp} = 81.8kg SEM 4.0, P_{Val} = 0.78), gender (m/f, ACT_{Grp} = 13/5, HMS_{Grp} = 17/3, P_{Val} = 0.44), BV (ACT_{Grp} = 5415ml SEM 337, HMS_{Grp} = 5363ml SEM 300, P_{Val} = 0.91), Hct (ACT_{Grp} 43.6% SEM 0.7, HMS_{Grp} = 41.9% SEM 0.8, P_{Val} = 0.12), PTr (ACT_{Grp} = 1.0 SEM 0.02, HMS_{Grp} = 1.0 SEM 0.02, P_{Val} = 0.51), APTTr (ACT_{Grp} = 0.9 SEM 0.02, HMS_{Grp} = 0.9 SEM 0.02, P_{Val} = 0.44) and plt (ACT_{Grp} = 213x10^3/ml SEM 8.4, HMS_{Grp} = 235x10^3/ml SEM 18.0, P_{Val} = 0.27) (table 11.3).

Similarly, operative data was comparable for; CPB time (ACT_{Grp} = 90min SEM 10.1, HMS_{Grp} = 102min SEM 9.3, P_{Val} = 0.43), ACC time (ACT_{Grp} = 67min SEM 8.5, HMS_{Grp} = 72min SEM 8.4, P_{Val} = 0.65), minimum CPB temperature (ACT_{Grp} = 31.8^{0}C SEM 0.3, HMS_{Grp} = 31.4^{0}C SEM 0.5, P_{Val} = 0.52), max re-warm temperature (ACT_{Grp} = 37.1^{0}C SEM 0.04, HMS_{Grp} = 37.1^{0}C SEM 0.06, P_{Val} = 0.68), CPB clear fluid volume (crystalloid and colloid)(ACT_{Grp} = 5187ml SEM 378, HMS_{Grp} = 4969ml SEM 285, P_{Val} = 0.68)*, dilution factor (CPB clear fluid volume/BV) (ACT_{Grp} = 1.1 SEM 0.2, HMS_{Grp} = 1.0 SEM 0.1, P_{Val} = 0.64) and Aprotinin administration(y/n, ACT_{Grp} = 7/18, HMS_{Grp} = 11/20, P_{Val} = 0.61) (table 11.3).

*This figure includes the 2250ml extra-corporeal circuit prime.

Table (11.3) *Patient and operative characteristics. ACT = activated clotting time, HMS = heparin management system group, P_{Val} = P value, Hb = haemoglobin concentration, Hct = haematocrit, PTr = pro-thrombin time ratio, APTTr = activated partial thromboplastin time ratio, Plt = platelet count, CPB = cardio-pulmonary bypass and ACC = aortic cross clamp. All data is presented as mean (SEM).*

	ACT_{Grp} (n = 18)	**HMS_{Grp} (n = 20)**	**P_{Val}**
Patient Variables			
Age (y)	60.4 (2.8)	64.8 (2.3)	0.24
Gender (m/f)	13/5	17/3	0.44
Weight (kg)	83.5 (4.6)	81.8 (4.0)	0.78
Blood Volume (ml)	5415 (337)	5363 (300)	0.91
Pre-operative haematology			
Hct (%)	43.6 (0.7)	41.9 (0.8)	0.12
PTr	1.0 (0.02)	1.0 (0.02)	0.51
APTTr	0.9 (0.02)	0.9 (0.02)	0.44
Plt (x10^3/ml)	213 (8.4)	235 (18.0)	0.25
CPB Data			
CPB Time (min)	90 (10.1)	102 (9.3)	0.43
ACC Time (min)	67 (8.5)	72 (8.4)	0.65
Min Temp (^{0}C)	31.8 (0.3)	31.4 (0.5)	0.52
Max Temp (^{0}C)	37.1 (0.04)	37.1 (0.06)	0.68
CPB clear fluid volume (ml)*	5187 (378)	4969 (285)	0.68
Dilution Factor	1.1 (0.2)	1.0 (0.1)	0.64
Aprotinin (y/n)	7/18	11/20	0.61

* This figure includes the 2250ml extra-corporeal circuit prime.

Table (11.4) *Heparin and protamine administration. ACT = activated clotting time, HMS = heparin management system and P_{Val} = P value. All data is presented as mean (SEM).*

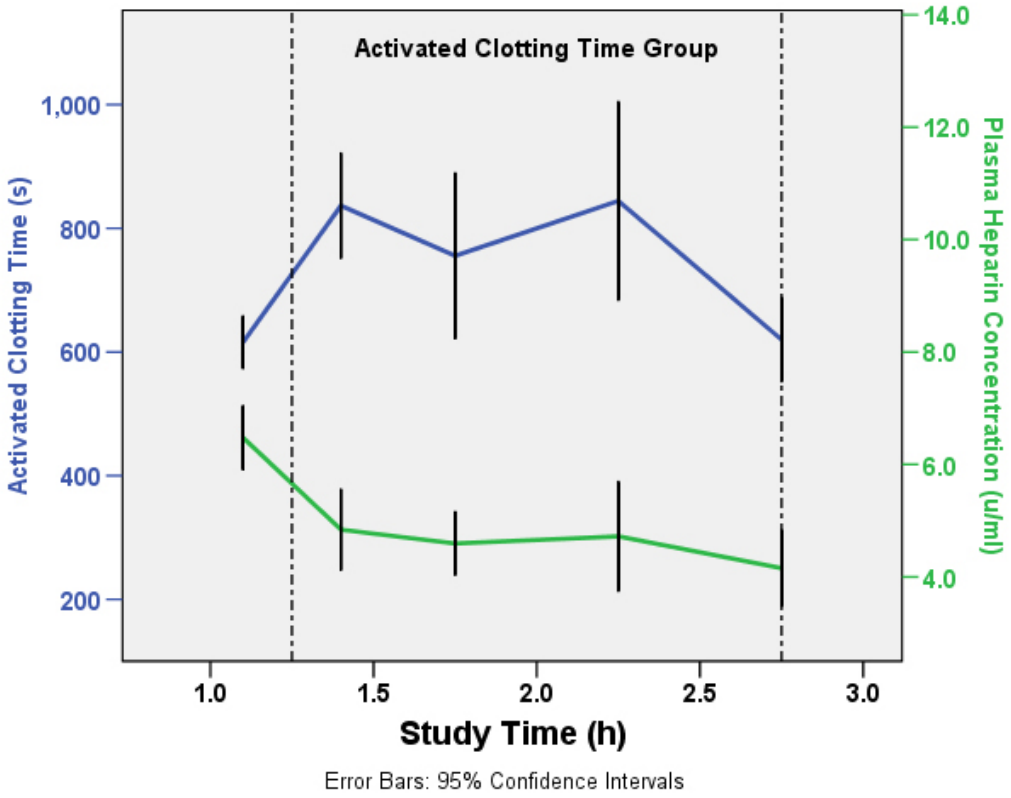
	Heparin (IU)			**Protamine (mg)**		
	ACT_{Grp} (n = 18)	**HMS_{Grp} (n = 20)**	**P_{Val}**	**ACT_{Grp} (n = 18)**	**HMS_{Grp} (n = 20)**	**P_{Val}**
Initial Dose	25056 (1370)	25164 (1808)	0.96	253 (20)	233 (14)	0.43
Additional Dosage	17038 (1328)	26373 (2309)	0.001	37 (4)	42 (6)	0.55
Total Dose	42094 (2167)	51537 (3715)	0.04	290 (22)	274 (14)	0.56
Total per kg of Body Weight	510 (15)	633 (32)	0.001	3.5 (0.2)	3.4 (0.2)	0.70

11.4.2 Heparin and Protamine Administration

Although no statistically significant difference was observed in the initial heparin dose administered for the two groups (ACT_{Grp} = 25056IU SEM 1370, HMS_{Grp} = 25164IU SEM 1808, P_{Val} = 0.96), HMS_{Grp} received a greater total dose (ACT_{Grp} = 42094IU SEM 2167, HMS_{Grp} = 51537IU SEM 3715, P_{Val} = 0.04). All patients reached the target ACT of 480s in ACT_{Grp} (n = 18/18) while two patients required a further heparin bolus prior to CPB in HMS_{Grp} (n = 2/20) (table 11.4).

The initial protamine dose administered was comparable between the two groups (ACT_{Grp} = 253mg SEM 20, HMS_{Grp} = 233mg SEM 14, P_{Val} = 0.43) with complete reversal achieved in 12 patients (n = 12/18) in ACT_{Grp} and 16 patients (n = 16/20) in HMS_{Grp}. Heparin rebound was identified in 5 patients (n = 5/18) in ACT_{Grp} and 4 patients (n = 4/20) in HMS_{Grp} following ICU_{Ret} (table 11.4).

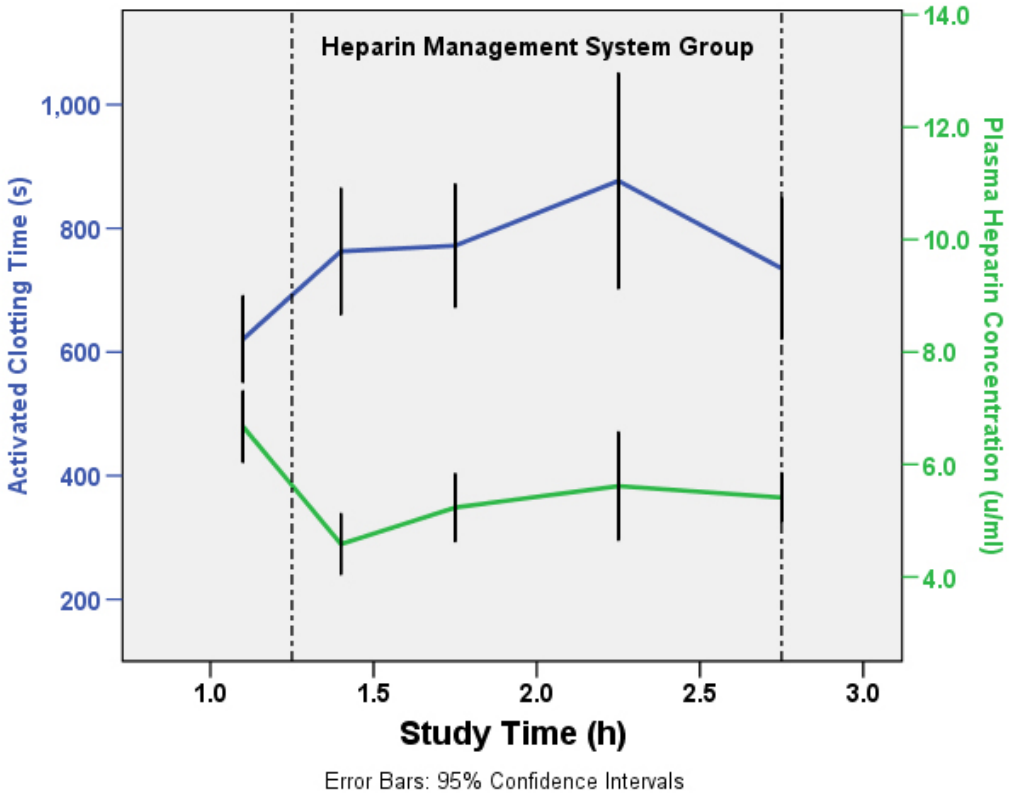
Figure (11.2) *ACT and PH_{Conc} during CPB for ACT_{Grp}. Time = 1.1h equates to 5min post-heparin. Hashed lines indicate period of CPB. ACT = activated clotting time, PH_{Conc} = plasma heparin concentration and CPB = cardio-pulmonary bypass.*



11.4.3 Activated Clotting Time and Plasma Heparin Concentration

ACT rose markedly in both groups from its baseline value at t = 0 (ACT_{Grp} = 141s SEM 3.5, HMS_{Grp} = 138s SEM 2.7, P_{Val} = 0.46) following the administration of the initial heparin bolus (ACT_{Grp} = 616s SEM 21.1, HMS_{Grp} = 621s SEM 33.0, P_{Val} = 0.89). At t = 10min CPB a further rise occurred (ACT_{Grp} = 837s SEM 40.1, HMS_{Grp} = 763s SEM 48.3, P_{Val} = 0.25) prior to a gradual fall towards the end of CPB. Although this fall was more marked in ACT_{Grp} a statistically significant difference did not exist (ACT_{Grp} = 620s SEM 32.4, HMS_{Grp} = 735s

Figure (11.3) *ACT and PH_{Conc} during CPB for HMS_{Grp}. Time = 1.1h equates to 5min post-heparin. Hashed lines indicate period of CPB. ACT = activated clotting time, PH_{Conc} = plasma heparin concentration, CPB = cardio-pulmonary bypass and HMS = heparin management system.*



SEM 54.0, P_{Val} = 0.07) (figures 11.2 and 11.3).

PH_{Conc} fell for both groups from t = 5min post-heparin (ACT_{Grp} = 6.5u/ml SEM 0.3, HMS_{Grp} = 6.7u/ml SEM 0.3, P_{Val} = 0.63) following the start of CPB (ACT_{Grp} = 4.8u/ml SEM 0.3, HMS_{Grp} = 4.6u/ml SEM 0.3, P_{Val} = 0.56). Thereafter an overall rise was noted in HMS_{Grp} with a fall in ACT_{Grp} at t = end-CPB (ACT_{Grp} = 4.2u/ml SEM 0.3, HMS_{Grp} = 5.4u/ml SEM 0.2). Although the difference between ACT_{Grp} and HMS_{Grp} was significant (P_{Val} = 0.003) for this time point, all other time points were non-significant (figures 11.2 and 11.3).

No statistically significant difference in ACT existed at t = 5m post-protamine (ACT_{Grp} = 141s SEM 3.5, HMS_{Grp} = 134s SEM 2.8, P_{Val} = 0.10). This finding was also similar for PH_{Conc} (ACT_{Grp} = 0.3u/ml SEM 0.1, HMS_{Grp} = 0.2u/ml SEM 0.1, P_{Val} = 0.50). At t = ICU_{Ret} data was comparable between the groups for both ACT (ACT_{Grp} = 143s SEM 6.3, HMS_{Grp} = 143s SEM 4.5, P_{Val} = 0.97) and PH_{Conc} (ACT_{Grp} = 0.2u/ml SEM 0.1, HMS_{Grp} = 0.2u/ml SEM 0.1, P_{Val} = 0.73) (table 11.5).

11.4.4 Peri-operative Coagulation

No statistically significant difference existed at t = 0 between the two groups for; Hct (ACT_{Grp} = 41.2% SEM 1.0, HMS_{Grp} = 40.1% SEM 1.0, P_{Val} = 0.40), PTr (ACT_{Grp} = 1.0 SEM 0.01, HMS_{Grp} = 1.0 SEM 0.02, P_{Val} = 0.15), APTTr (ACT_{Grp} = 1.0 SEM 0.02, HMS_{Grp} = 1.0 SEM 0.02, P_{Val} = 0.30) and platelet count (Plt) (ACT_{Grp} = 196x10^3/ml SEM 9.0, HMS_{Grp} = 201x10^3/ml SEM 18.5, P_{Val} = 0.81). This was also noted at t = 5m post-protamine for; Hct (ACT_{Grp} = 26.8% SEM 0.8, HMS_{Grp} = 26.8% SEM 0.12, P_{Val} = 0.99), APTTr (ACT_{Grp} = 1.7 SEM 0.08, HMS_{Grp} = 1.5 SEM 0.08, P_{Val} = 0.24) and platelet count (ACT_{Grp} = 92 x103/ml SEM 9.9, HMS_{Grp} = 99 x103/ml SEM 7.7, P_{Val} = 0.59). However, a significant difference was noted for PTr at this time point (ACT_{Grp} = 1.7 S SEM 0.04, HMS_{Grp} = 1.5 SEM 0.06, P_{Val} = 0.03). At t = ICU_{Ret}, all the variables of; Hct (ACT_{Grp} = 28.1% SEM 1.0, HMS_{Grp} = 26.8% SEM 1.3, P_{Val} = 0.43), PTr (ACT_{Grp} = 1.3 SEM 0.05, HMS_{Grp} = 1.2 SEM 0.03, P_{Val} = 0.42), APTTr (ACT_{Grp} = 1.7 SEM 0.16, HMS_{Grp} = 1.5 SEM 0.08, P_{Val} = 0.34) and Plt (ACT_{Grp} = 107x10^3/ml SEM 9.8, HMS_{Grp} = 118x10^3/ml SEM 10.3, P_{Val} = 0.46) showed no significant difference (table 11.6).

Table (11.5) *ACT and PH_{Con} following protamine administration. ACT = activated clotting time, PH_{Con} = plasma heparin concentration, HMS = heparin management system, PH_{Con} = plasma heparin concentration, ICU = intensive care unit and P_{Val} = P value. All data is presented as mean (SEM).*

	End-CPB			**P**ost-protamine			**I**CU$_{Ret}$		
	ACT$_{Grp}$ (n = 18)	**H**MS$_{Grp}$ (n = 20)	**P**$_{Val}$	**A**CT$_{Grp}$ (n = 18)	**H**MS$_{Grp}$ (n = 20)	**P**$_{Val}$	**A**CT$_{Grp}$ (n = 18)	**H**MS$_{Grp}$ (n = 20)	**P**$_{Val}$
ACT (s)	620 (32.4)	735 (54.0)	0.07	141 (3.5)	134 (2.8)	0.10	143 (6.3)	143 (4.5)	0.97
PH_{Con} (u/ml)	4.2 (0.3)	5.4 (0.2)	0.003	0.3 (0.1)	0.2 (0.1)	0.5	0.2 (0.1)	0.2 (0.1)	0.73

Table (11.6) *Peri-operative coagulation. ICU = intensive care unit, ACT = activated clotting time, HMS = heparin management system, P_{Val} = P value, Hct = haematocrit, PTr = pro-thrombin time ratio, APTTr = activated partial thromboplastin time ratio and Plt = platelet count. All data is presented as mean (SEM).*

	Induction			Post-Protamine			ICU_{ret}		
	ACT_{Grp} (n = 18)	**H**MS_{Grp} (n = 20)	**P**$_{Val}$	**A**CT_{Grp} (n = 18)	**H**MS_{Grp} (n = 20)	**P**$_{Val}$	**A**CT_{Grp} (n = 18)	**H**MS_{Grp} (n = 20)	**P**$_{Val}$
Hct (%)	41.2 (1.0)	40.1 (1.0)	0.40	26.8 (0.8)	26.8 (1.2)	0.99	28.1 (1.0)	26.8 (1.3)	0.43
PTr	1.0 (0.01)	1.0 (0.02)	0.15	1.7 (0.04)	1.5 (0.06)	0.03	1.3 (0.05)	1.2 (0.03)	0.42
APTTr	1.0 (0.02)	1.0 (0.02)	0.30	1.7 (0.08)	1.5 (0.08)	0.24	1.7 (0.16)	1.5 (0.08)	0.34
Plt *(x10^3/ml)*	196 (9.0)	201 (18.5)	0.81	92 (9.9)	99 (7.7)	0.59	107 (9.8)	118 (10.3)	0.46

Table (11.7) *Haemostatic markers. ACT = activated clotting time, HMS = heparin management system, P_{Val} = P value, TAT = thrombin anti-thrombin complex and AT-III = anti-thrombin III. All data is presented as mean (SEM).*

	ACT$_{Grp}$	**H**MS$_{Grp}$	**P**$_{Val}$
Pre-CPB			
TAT (mg/l)	12.5 (3.7)	12.0 (3.6)	0.94
D-Dimer (ng/ml)	956 (92)	1135 (162)	0.33
Fibrinogen (g/l)	2.8 (0.1)	3.4 (0.2)	0.04
AT-III (IU/ml)	0.84 (0.03)	0.88 (0.04)	0.50
End-CPB			
TAT (mg/l)	16.3 (2.9)	22.3 (6.6)	0.39
D-Dimer (ng/l)	1318 (300)	1981 (982)	0.50
Fibrinogen (g/l)	1.8 (0.2)	1.6 (0.1)	0.33
AT-III (IU/ml)	0.50 (0.03)	0.51 (0.03)	0.91

11.4.5 Thrombin Generation, Fibrinolysis and Antithrombin III

At t = 0, all the variables of; TAT (ACT$_{Grp}$ = 12.5mg/l SEM 3.7, HMS$_{Grp}$ = 12.0mg/l SEM 3.6, P$_{Val}$ = 0.94), d-dimer (ACT$_{Grp}$ = 956ng/ml SEM 92, HMS$_{Grp}$ = 1135ng/ml SEM 162, P$_{Val}$ = 0.33) and AT-III (ACT$_{Grp}$ = 0.84IU/ml SEM 0.03, HMS$_{Grp}$ = 0.88IU/ml SEM 0.04, P$_{Val}$ = 0.50), with the exception of fibrinogen (ACT$_{Grp}$ = = 2.8g/l SEM 0.1, HMS$_{Grp}$ = 3.4g/l SEM 0.2, P$_{Val}$ = 0.04), were comparable. At t = end-CPB, no significant difference existed between the variables of; TAT (ACT$_{Grp}$ = 16.3mg/l SEM 2.9, HMS$_{Grp}$ = 22.3mg/l SEM 6.6, P$_{Val}$ = 0.39), d-dimer (ACT$_{Grp}$ = 1318ng/ml SEM 300, HMS$_{Grp}$ = 1981ng/ml SEM 982, P$_{Val}$ = 0.5), fibrinogen (ACT$_{Grp}$ = 1.8g/l SEM 0.2, HMS$_{Grp}$ = 1.6g/l SEM 0.1, P$_{Val}$ = 0.33) and AT-III (ACT$_{Grp}$ = 0.50IU/ml SEM 0.03, HMS$_{Grp}$ = 0.51IU/ml SEM 0.03, P$_{Val}$ = 0.91) (table 11.7).

11.4.6 Post-operative Drain Loss and Blood Product Requirement

For 12h drain loss no significant difference was noted between the groups (ACT_{Grp} = 442ml SEM 76, HMS_{Grp} = 637ml SEM 93, P_{Val} = 0.06). This was also mirrored in the 24h RBC transfusion data (ACT_{Grp} = 1.1u SEM 0.3, HMS_{Grp} = 1.6u SEM 0.5, P_{Val} = 0.47). An insufficient number of patients received clotting factors to allow meaningful analysis.

11.5 Summary of Principle Findings

Demographic and peri-operative data was comparable for both groups. Similarly, no difference was observed in initial patient heparin bolus between ACT_{Grp} and HMS_{Grp}. HMS_{Grp} did receive a significantly greater total dose as a result of the additional heparin administration during CPB. No difference was observed in protamine administration in terms of, initial, additional and total dosage. Although ACT did not differ between the two groups, PH_{Conc} was significantly higher in HMS_{Grp} at t = end-CPB. Haematological investigation revealed no difference in Hct, APTTr and platelet count at induction, post-protamine and ICU_{Ret}. Only PTr demonstrated a significant difference at t = 5m post-protamine. No difference existed between, TAT, d-dimer and AT-III at t = end-CPB. As fibrinogen was significantly lower in HMS_{Grp} prior to CPB this parameter was not suitable for valid comparison. Chest drain losses at 12h and 24h RBC transfusion total post-operatively were again, not significantly different.

Please see section 12.9 (page - 165) for a full discussion of the above findings.

Part III

Discussion, Conclusions and Future Research

Chapter 12

Discussion

12.1 Patient and Procedural Variables Associated with Red Cell Transfusion

Study Aim

- *To retrospectively analyse what patient and operative variables are associated with RBC transfusion.*

Please see section 3.5 (page - 52) for a summary of the principle findings.

Advancing age has been established as a predictor of RBC transfusion requirement by many studies, a finding confirmed by our data[131–141]. Primarily this is thought to relate to a reduction in renal function and therefore erythropoietin secretion[188]. Another consideration with advancing age is the increasing friability of soft tissues and the resultant predisposition to surgical blood loss[174;189].

Age is not only related to pre-operative [Hb] but also body size. This study identified strong associations between; gender, height, weight and BSA with RBC transfusion. An interesting observation is that all the aforementioned variables may be considered determinants of pre-operative RCV (equations A.2 and A.3[171]. Several studies have identified these parameters as being predictors of RCV loss and therefore transfusion requirement[131–141]. An alternative angle may be to consider the ability to tolerate RCV loss. Those with a higher RCV pre-operatively will not suffer the same percentage reduction in RCV for a given RCV loss than those at the lower end of the spectrum.

When considering pre-operative morbidity, it was evident that those in poorer physical condition (diabetes mellitus, poor left ventricular function, pre-operative IABP, high pre-operative creatinine level, NYHA class and Euro score) were more likely to receive an RBC transfusion[190]. Several study limitations may explain this finding. As the timing of transfusion was not recorded, the RBCs administered may have been given at any time during the patients hospital stay. It seems likely that increasing co-morbidity will result in prolonged periods in the ICU, with resultant delay in hospital discharge, raising the incidence of transfusion events. It is also important to note that as this was a retrospective analysis,

there was no control over adherence to local RBC transfusion protocol (table 4.1). Therefore, sicker patients may simply receive more RBCs in the belief that it will improve outcome.

In addressing some of these co-morbid parameters individually, additional hypothesis may be offered. Diabetes mellitus has previously been identified as an important risk factor for RBC transfusion among those undergoing cardiac surgery. Possible explanations include more advanced CAD at the time of presentation and the presence of diabetic complications, both of which may increase transfusion risk for the reasons outlined above[191]. IABPs carry their own inherent risks for blood product requirement. Mechanical destruction of platelets through continued use and arterial trauma at the time of insertion may both predispose to bleeding[192;193]. Smoking status has been identified as a transfusion risk factor with non-smokers more likely to receive RBCs. One reason suggested has been the protective effects of secondary polycythaemia in smokers[194;195]. This study could not confirm these suggestions as no association with smoking status was found. Creatinine was only found to be associated with the number of units RBC transfused and not RBC transfusion status. Although it is hard to say if this was a significant finding, high creatinine has been associated in the past with an increase in peri-operative blood loss[196].

Surgical priority, redo status and Hardy class were all associated with RBC transfusion. Possible reasons may include prolonged CPB times and a greater likelihood of post-operative complications[138;139]. Patients with long CPB times are predisposed to an increase in coagulation activation and subsequently RCV loss[197]. Another consideration may be the artificial depression of [Hb] by the large clear fluid load invariably associated[127–129]. This is further borne out by the finding that patients undergoing CABG on-pump are more likely to receive RBCs[198;199].

It is evident that the majority of the positive indicators for RBC transfusion found in this study may be associated with peri-operative RCV loss. However, other possible rationales for an associated increase in transfusion requirement with these variables were apparent. Parameters associated with a small pre-operative RCV may predict an individual's ability to cope with RCV loss. Furthermore, small RCV in the presence of CPB associated haemodilution may dictate that

patients are transfused despite little actual RCV loss. In the following section (12.2) we discuss the results of a study to challenge this hypothesis in which the association of several key variables with RCV loss and gain was examined.

12.2 Factors Predicting Loss and Gain of Red Cell Volume

Study Aim

- *To determine what pre and intra-operative variables are associated with loss or gain in RCV due to bleeding or RBC transfusion.*

Please see section 4.5 (page - 65) for a summary of the principle findings.

Our results demonstrated that; age, sex, height, weight, BSA and initial Hct are all associated with RBC transfusion but not with RCV loss. These variables are all predictors of pre-operative RCV as outlined by the ICSH (equations A.2 and A.3)[171]. Thus it would appear that those patients with a low RCV are not at greater risk of RCV loss but may be less able to cope with any loss that does occur[131;136]. Adding further weight to this argument was the finding that percentage reduction in initial RCV (but not absolute RCV loss) was associated with RBC transfusion. It is also likely that these individuals are more prone to the 'dilutional anaemia' encountered during cardiac surgery[139;141]. As described in previous predictive models, our results suggest that the above variables are all indicators of transfusion risk when that decision is solely based upon a [Hb] threshold[131–141]. However, they do not predict the volume of RCV a patient is likely to lose as has often been the assumption.

None of the operative variables studied of; CPB time, ACC time and theatre fluid administration had any association with post-operative RCV loss or RBC gain. Studies have also identified these factors as predictors for transfusion through a combination of small BSA, the dilutional effects of the CPB circuit and the large volumes of exogenous fluid administered[135–139;141]. Although our

results did not demonstrate any such association it is clear that individuals with a small BSA are at a greater risk of receiving RBCs. Similarly, hypothermia has been identified as a causative factor for transfusion requirement due to its effects on haemostasis[174]. Although our study showed no association with RCV loss the explanation may simply be that as our cohort consisted of elective patients only there was little variability in minimum CPB temperature. Only a minimal difference was observed in RCV loss between those taking anti-platelet therapy within seven days of operation and those not.

The results from this study suggest that pre-operative RCV and haemodilution are key components in determining RBC transfusion when [Hb] is the sole parameter considered. Currently, much debate exists over what should be considered a safe transfusion threshold in the general and cardiac ICU. A growing body of evidence is beginning to suggest that lower [Hb] concentrations are well tolerated thus confirming clinical experience with Jehovah's witness patients over many years[6;114;137;200]. Thus the development of an RCV transfusion guideline, where lower [Hb]s are tolerated provided haemodilution is the predominant factor, may be the most effective way of rationalising the current liberal transfusion practice in cardiac surgery.

In the following sections we go on to discuss several key questions, outlined below, that will allow us to develop an RCV transfusion guideline;

- What is the relative contribution of haemodilution/ RCV loss to the post-operative anaemia encountered in cardiac surgery (section 12.3)?
- Is it possible to accurately measure RCV, reproducibly and reliably (section 12.4)?
- Will [Hb] recover as excess body water is shed following surgery (section 12.5)?
- What is the lowest [Hb] consistent with maintaining the minimal requirement for oxygen delivery peri-operatively (section 12.6)?

12.3 Peri-operative Red Cell, Plasma and Blood Volume Change

Study Aim

- *To determine the relative contributions of RCV loss and haemodilution to the post-operative anemia encountered following cardiac surgery.*

Please see section 5.5 (page - 76) for a summary of the principle findings.

The results obtained in this study strongly suggest that PV expansion is a significant factor in triggering RBC transfusion if this decision is based upon [Hb] alone. Three distinct peaks of PV expansion occurred at anaesthetic induction; end of CPB and at 16h of ICU management. General anaesthesia is known to cause a degree of systemic vasodilation resulting in PV expansion[201;202]. This is further compounded by the systemic inflammatory response resulting from the use of extra-corporeal circulation[203;204]. In order to maintain circulatory volume during this phase large volumes of clear fluids are usually administered, resulting in profound haemodilution[139;205]. During the initial hours of ICU stay patients vasodilate further as they re-warm fully to normothermia, again resulting in more clear fluid administration[206]. At its greatest, PV reached one and a half times its pre-operative value.

When considering RCV, the greatest loss occurred during CPB due to residual volume remaining within the extra-corporeal circuit. It should be noted that approximately half of this was returned to the patient in the from of 'pump blood'. The most interesting observation, however, occurred during the initial phase of ICU management. Paralleling the rise in PV described above, patients were transfused RBCs to the extent of a net RCV gain, despite the loss of RCV in the thoracic effluent. Although our departments transfusion protocol threshold [Hb] rises from 8g/dl to 9g/dl at 4h post-operatively, this trend continued well beyond this time point and can therefore not be explained by this change. Indeed three patients had an estimated RCV at 24h in excess of the pre-operative value.

The results of this study suggest that transfusing RBCs based upon [Hb] is an inaccurate way of replacing RCV loss. This is primarily due to the artificial depression of [Hb] through PV expansion[139;201–206]. In tandem with the results previously discussed (section 12.2), three key points now appear evident;

1. RCV is relatively well preserved in patients undergoing cardiac surgery.
2. Pre-operative RCV is the principle determinant of ability to tolerate RCV loss.
3. When transfusion is based upon [Hb] alone, PV expansion has a profound effect on the decision to administer RBCs and may lead to over-transfusion.

Taken together, these findings provide the basis for the development of an RCV based transfusion guideline as outlined in section 12.2.

Although we consider the above statements to be valid, it is important to remember they are based upon mechanical estimations of blood loss. That is to say, calculation of RCV loss is by swab weight, drain loss etc and the measured Hct. Such techniques are prone to a certain amount of error and can often only be considered 'best guess'. Until recently, accurate repeatable RCV measurement has not been possible due to the radioactive nature of the markers used. In the following section (12.4) we discuss the evaluation of a new and repeatable technique using NaF flow cytometry in the calculation of RCV[130;148;176].

12.4 Evaluation of Sodium Fluorescein Flow Cytometry in the Determination of Red Cell Volume

Study Aim

- *To assess the accuracy of NaF flow cytometry in the measurement of changes in RCV by PIHD.*

Please see section 6.5 (page - 82) for a summary of the principle findings.

Accurate replacement of RCV is dependant upon a technique which allows its direct measurement. Such an investigation should be; simple to perform, non time consuming, non-toxic and above all repeatable within a short time frame. NaF labelled RBCs offer the first opportunity for a repeatable test that can directly quantify the degree of surgical RCV loss[130;148;176]. The results of the preliminary investigation we performed indicated that the rate of decay in fluorescence of the NaF-RBCs was to rapid to allow the meaningful clinical application of this technique. Paradoxically, it is this very property that allows for the repeat application of this process within a short time frame. The original application by Lauermann involved injection and analysis of results within a short time frame ($<$ 20min). Despite this, the good association demonstrated with Cr^{51} labelled RBCs is perhaps surprising given that our results documented a fluorescent RBC half life of 20min[148;176].

As a result of the above, the derivation of the RCV transfusion guideline, described in this thesis (appendix C, page - 185), was based upon the mechanical methodology described previously in chapters 4 (page - 53) and 5 (page - 66).

In section 14.2 (page - 174), we suggest a method of correcting the deficiencies outlined above to allow for the proper assessment of NaF flow cytometry in detecting RCV change.

12.5 The Effect of Gain in Total Body Water on Haemoglobin Concentration and Body Weight

Study Aim

- *To determine whether [Hb] will recover as excess TBW is shed post-operatively in association with a reduction in body weight.*

Please see section 7.5 (page - 93) for a summary of the principle findings.

At all times, the transfusion threshold of 8g/dl post-operatively was rigidly adhered to. All patients receiving RBCs after day 1 were excluded. This has effectively created a level 'playing field' allowing us to compare TBW, body weight and [Hb] in a meaningful way. The first observation of note was the recovery of [Hb] in association with a reduction in TBW until day 5 but not between days 5 and 10. One possible explanation for this finding may lie within the results discussed in section 12.3. It was observed that both BV and PV rose markedly, artificially depressing [Hb][129;173]. The suggestion from this study may be that TBW acts as a surrogate marker for PV. As PV, and therefore TBW, returns to its pre-operative state the dilutional effect on [Hb] is lost. From the trend observed in chapter 5 (page - 66) (a progressive fall beginning at 16h post-operatively), and these results, it would seem reasonable to postulate that normal circulatory physiology is restored around day 5. The rise in [Hb] beyond this point may be explained by the recovery of haemopoietic bone marrow function[207;208].

The finding that body weight fell in association with reduction in TBW and [Hb] recovery, between days 1 and 5, suggests that simple monitoring of body weight may act as a guide to an individual's fluid status, and therefore scope for [Hb] recovery. Indeed, this confirms what has been observed in clinical practice for many years (and prompted this study). Although the study period between days 1 and 3 was non-significant for body weight and [Hb], some strength of association was identified.

12.5 The Effect of Gain in Total Body Water on Haemoglobin Concentration and Body Weight

The final finding of note was that although body weight returned to near its pre-operative value at day 5, TBW remained significantly elevated, presumably as a consequence of the highly catabolic effects of cardiac surgery[178]. Thus, if using body weight to assess an individual's fluid status, a value lower than that recorded pre-operatively should be sought.

As mentioned in the introductory chapter 1 (page - 2), it has previously been assumed that higher [Hb]s are required in order to maintain adequate systemic oxygenation peri-operatively. This suggests that lower [Hb]s are not tolerated. The evidence we have gathered thus far contradicts this assumption. We have demonstrated that post-operative anaemia may be considered largely dilutional and so may partially correct over a short period as fluid is lost. Taken in tandem with the results of haemodilution studies (section 1.5.3.1, page - 31), it appears that this is an entirely patho-physiological state where a variety of compensatory mechanisms act to maintain adequate systemic oxygenation up to an as yet undefined critical point. Of the questions identified in section 12.2, only one remains in order to complete the body of evidence required to develop an RCV based transfusion guideline.

12.6 Oxygen Delivery and Haemoglobin Concentration in Cardiac Surgery

Study Aim

- *To examine the relationship between actual measured oxygen delivery and the corresponding estimated critical values peri-operatively.*

Please see section 8.5 (page - 105) for a summary of the principle findings.

The results obtained demonstrate that lowering core body temperature significantly reduces both DO_{2Crit} and VO_2. As patients are re-warmed towards normothermia both the aforementioned variables rise while DO_2 remains relatively fixed. Discontinuation of CPB, and restoration of the cardiac based circulation, allows DO_2 to rise by way of an increase in BFI. Consequently, at no time did DO_2 significantly approach DO_{2Crit}. This suggests that anaerobic metabolism is of minimal importance in post-operative lactate accumulation following CPB. Calculated [Hb_{Crit}] suggested that values as low as 3.9g/dl during CPB (provided CPB flow rates are increased on re-warming), and 5.4g/dl post-operatively could theoretically be tolerated.

Oxygen debt accrual during CPB has long been used as the basis for any argument pertaining to maintaining a higher peri-operative [Hb]. Principally this states that pathological supply dependency of VO_2 on DO_2 below a critical point results in anaerobic metabolism and lactate accumulation[89]. Current evidence suggests that this may not be the case. The observed dependency in many cases may simply be a manifestation of mathematical coupling whereby many of the of the parameters used in the calculation of VO_2 are also used for DO_2[91;92]. This theory is strongly supported by the finding that VO_2 measured by the reverse Fick principle has no association with VO_2 as measured by expired gas analysis (section 1.5.1.1, page - 23)[93].

Consideration also has to be given to causative factors of lactate accumulation, as described fully in section 1.5.1.3 (page - 26). Outwith anaerobic metabolism; lactate washout from hypo-perfused tissues, a reduction in lactate clearance and deactivation of PDH by insulin and calcium containing drugs may all play a role[89;101;102]. More recently attention has turned to the large volumes of exogenous fluid administered during CPB. The net result of such a large non-[HCO3$^-$] containing fluid load may be to reduce extra-cellular [HCO3$^-$] and therefore an individual's buffering capacity. This may be exacerbated where fluids with little or no strong ion difference are given e.g. normal saline. The net effect may be to increase hydrogen ion dissociation with a resultant metabolic acidosis[99;103;104].

Although this study was largely a theoretical mathematical construct based upon real time patient observations, the recording of such large differences in observed and calculated critical values suggests that current transfusion practice is excessive if oxygen requirement is the sole parameter considered. In association with the evidence discussed so far we can now hypothesise the following;

- The ability to tolerate RCV loss is determined by the pre-operative RCV (section 12.2).
- Haemodilution is a significant factor in determining the transfusion of RBCs when [Hb] is the sole parameter considered (section 12.3).
- Haemodilution is maximal at 16h post-operatively, resolving by day 5 (section 12.5).
- A significant oxygen/glshb reserve exists peri-operatively (section 12.6).

The above provided the basis for the construction of the RCV based transfusion guideline described in appendix C (page - 185).

12.7 The Impact of a Red Cell Volume Based Transfusion Guideline on Blood Usage and Clinical Outcome

Study Aim

- *To determine if RBC transfusion based upon an estimate of patient RCV confers benefit in terms of a reduction in blood usage and improved clinical outcome.*

Please see section 9.6 (page - 121) for a summary of the principle findings.

The clinical application of the RCV based guideline, in a pilot clinical study, resulted in a significant reduction in blood usage expressed as both % patients transfused and UPPO. When considering the annual cardiac case load in our unit of 850 patients per annum, the assumed saving in RBCs transfused would approximate 500u. This represents half the number of RBCs currently prescribed. Also of importance was the finding that all outcome parameters, with the exception of hospital stay (reduced in RCV_{Grp}), were comparable between the two groups. However, it should be noted that this study was not powered to detect a difference for any of these variables. Furthermore, as this study was non-blinded an element of bias may have existed.

As more rational transfusion policies become more frequently implemented, it seems certain that physiological factors, additional to [Hb], will be given increasing consideration in deciding when to transfuse RBCs[142;143]. Unfortunately, such a scenario can only apply to those individuals with a relatively stable blood balance e.g. general ICU patients. Further consideration is required for those undergoing an operative procedure who will inevitably loose RCV of varying degree. The RCV guideline used in this study provided an estimate of an individual's ability to tolerate RCV loss while giving greater relevance to post-operative [Hb]. The results obtained demonstrate that the use of such an approach, while significantly

reducing RBC transfusion, may have no significant bearing on patient outcome albeit for the limited number of outcome variables examined.

In section 14.1 (page - 173), we propose a study that will more accurately evaluate the role of an RCV guideline in post-operative outcome.

12.8 Lung Injury Following Red Cell Transfusion

Study Aim

- *To determine the association between pulmonary endothelial injury and RBC transfusion.*

Please see section 10.5 (page - 131) for a summary of the principle findings.

ALI is a major cause of morbidity following surgery utilising extra-corporeal circulation[70–72]. Although RBC transfusion has been implicated in the severe forms of this condition (TRALI), uncertainty exists as to its contribution in subtler forms[74]. Primarily, this is attributable to the non-specificity of the inflammatory markers usually studied[151]. In addressing this issue we have studied the role of exhaled NO in quantifying the degree of pulmonary endothelial damage following RBC transfusion[152–154].

The results of our analysis were largely disappointing in relation to the data gained. Only small numbers could be successfully studied due to the inability of many patients to complete the exhaled NO analysis at 48h. As a result no significant difference in RBC transfusion was present between the two study groups. The study cohort was therefore analysed as transfused versus non transfused groups. A further analysis was performed to correlate % NO reduction with the study variables for the full study cohort. Perhaps not surprisingly, no positive associations were found.

In section 14.3 (page - 175), we use the lessons learned from the initial pilot investigation to suggest a study that will properly address the study aim above.

12.9 Improving Anti-coagulation Strategies for Cardio-pulmonary Bypass

Study Aim

- *To evaluate the impact of both HMS and modified ACT based anti-coagulation management on; coagulation, thrombin generation, fibrinolysis, AT-III, post-operative blood loss and blood product requirement.*

Please see section 11.5 (page - 149) for a summary of the principle findings.

Thrombin generation during cardiac surgery may be triggered through several interlinked mechanisms. As blood is continually exposed to the foreign surfaces of the CPB circuit and subendothelial tissue within the chest cavity, factor XII may trigger the intrinsic or contact pathway. The interaction of factor VIIa and tissue factor similarly triggers the extrinsic pathway[197]. Causative factors include a combination of; sub-endothelial contact, an increase in tissue factor expression on mononuclear cell membranes or an increase in free plasma tissue factor through surgical trauma[209;210]. A key component of the above is considered to be the re-circulation of pericardial blood when it is returned to the CPB circuit[209;211].

To prevent the above, heparin is currently considered the anti-coagulant of choice for the purposes of CPB. Circulating free heparin has no direct anti-coagulant effect. However, when bound to AT-III it promotes the inactivation of thrombin, factor X and several serine proteases[212]. The dose of heparin required to achieve the above desired effect during CPB is far higher than for other indications e.g. deep venous thrombosis. This may relate to the exposure of blood to the foreign surface of the extra-corporeal circuit, promoting the formation of bound thrombin in addition to the free variety. As the anti-coagulant heparin/AT-III complex is a less potent inhibitor of bound thrombin, the requirement for heparin is significantly increased[165;213].

12.9 Improving Anti-coagulation Strategies for Cardio-pulmonary Bypass

In order to assess the adequacy of heparinisation for CPB, ACT remains the most widely used clinical test[158;160;168]. As mentioned in chapter 11 (page - 132), it has been shown that ACT does not correlate well with sensitive assays of PH_{Conc} during CPB. Possible explanations for this include the dilutional effects of the CPB circuit and the use of systemic hypothermia[162;163]. Another possible consideration is that as ACT is based upon contact activation (kaolin and/or cellite), it is not truly reflective of CPB conditions where the majority of thrombin generation is triggered by factor VIIa and tissue factor. The interaction of the above mechanisms may lead to an ACT that significantly overstates the PH_{Conc}, with sub-therapeutic anti-coagulation and thrombin generation[168]. To prevent this detrimental scenario, the use of HMSs has been championed in some quarters[164;166–169].

Prior to CPB, HMS provides in vitro analysis of individual HDR allowing patient specific administration of loading dose heparin prior to CPB. A heparin protamine titration technique is used during CPB to measure whole BH_{Conc}. This allows the early detection and correction of sub-therapeutic anti-coagulation. Previous research has highlighted the efficacy of this system both in preserving coagulation and reducing post-operative blood loss[164;166–169].

As our standard departmental policy is to administer heparin during CPB, independent of ACT, we hypothesised that similar results to that of an HMS may be achieved. This was indeed borne out in terms of; coagulation activation, post-operative blood loss and blood product requirement although it should be noted that the study was not powered to detect a true difference in the latter two variables. Despite the heparin dose received being significantly higher for HMS_{Grp}, this did not appear to influence the concentration of TAT complex. The answer may lie in comparison with other studies where the difference in heparin dose was appreciably larger between the two groups[164;166–169]. When considering protamine dosage, no significant difference was noted. The suggestion here was that ACT at termination of CPB is semi-accurate. Further evidence for this was noted in the observation that ACT fell on rewarming independent of PH_{Conc}.

In section 14.4 (page - 176), we suggest a study to investigate the role of continuous heparin infusions for CPB.

Chapter 13

Conclusions

13.1 Developing a Red Cell Volume Based Transfusion Guideline

RBC transfusion is a potentially life saving procedure with some of its earliest origins found on the battlefield[11]. From this acute trauma scenario we have arrived at today's liberal practice of transfusion based largely upon a [Hb] trigger. Much of the justification for this high blood usage in cardiac surgery has been based upon calculations of the oxygen kinetics required for a satisfactory outcome. However, there is an increasing recognition that the critical level of oxygen delivery required is way below that previously assumed while many of the aforementioned calculations are based upon mathematically questionable methodology[91;92;97]. Furthermore, no allowances have been made for the viscosity mediated patho-physiological adaptive responses to normovolaemic anaemia[112;113].

At present, the current literature does not provide a definitive answer to the question of when we should transfuse cardiac surgery patients? However, following recommendations by The American Society of Anesthesiologists in 1994, attitudes towards multiple physiological transfusion triggers have gradually changed[214]. Using such triggers in association with [Hb] is almost certainly of value in ICU patients with a stable blood balance. As this thesis has documented however, [Hb] alone is a poor indicator of peri-operative RCV where CPB is used. Thus a more multifactorial approach is required.

The first consideration in any transfusion decision should be to exclude any possibility of hypovolaemia[144]. Cardiac surgery patients may remain intravascularly deplete yet with a significant increase in TBW through compartmental fluid shifts (chapter 7, page - 83)[129;185]. Due consideration should be given to; relative hypotension (MAP $<$ 60mmHg), poor urine output ($<$ 0.5ml/kg/h), central venous pressure and pulmonary capillary wedge pressure (where available)[142;143]. Tachycardia, in the presence of optimal anaesthesia, has been cited as a key indicator of hypovolaemia[144]. However, as many cardiac surgery patients will have tachy or brady-arrythmias post-operatively this is likely to be an inaccurate sign[215]. It is also important to remember that patients may be haemodiluted

yet hypovolaemic due to expansion of the intra-vascular space[216]. Once normovolaemia has been established it would seem logical to adopt a two stage RBC transfusion strategy dependent upon the degree of haemodilution.

Prior to 48h due consideration should be given to; indicators of initial pre-operative RCV (and therefore the patients ability to tolerate RCV loss) (chapter 4, page - 53), the potential for excessive RCV loss, the relative contribution of haemodilution (chapter 5, page - 66) and indicators of an inadequate circulation or increase in oxygen requirement (chapter 8, page - 94)[129;144;173;177;185]. This would suggest that patients with a large initial RCV, in the presence of an adequate circulation and non-excessive RCV loss, should be transfused at a lower [Hb], as any fall is likely to relate haemodilution. The opposite scenario applies to those with a small initial RCV who should be transfused at a higher [Hb]. Again, any indicators of an inadequate circulation (in the presence of normovolaemia), or an increase in oxygen requirement, should act as a prompt to RBC transfusion (table 13.1)[144]. During this phase, cerebro-vascular disease (CVD) is likely to be a lesser consideration due to a viscosity mediated increase in cerebral blood flow rates[217]. This scenario may also apply to CAD, although this is likely to be of lesser importance still as patients will have had significant CAD excluded pre-operatively or undergone myocardial re-vascularisation as previously described[121].

Following 48h post-operatively, as the transient effects of hemodilution are lost, physiological and clinical factors, including [Hb], are likely to be of increasing importance in the decision to transfuse RBCs[129;173;177;185]. More emphasis should also be placed upon the presence of CVD and/or incomplete myocardial revascularisation.

In order to incorporate any such guidelines into everyday clinical practice, a simple easy to use decision making process is required. In chapter 9 (page - 106), we incorporated; patient weight (as an estimate of pre-operative RCV), [Hb], drain loss and several basic physiological criteria into such an algorithm. The results obtained demonstrate the efficacy of such a process in rationalising RBC transfusion. Although we consider the results reported in this study, and the proceeding chapters, to be valid a note of caution needs to be applied in several areas. In several of the chapters, multiple testing of the same dataset may have yielded positive results through chance. Furthermore, the algorithm in chapter 9

(page - 106) was trialled by non-blinded RCT and was underpowered to detect any true difference in clinical outcome. For this reason, further appropriately blinded and powered research is required before any definitive conclusions can be drawn as proposed in section 14.1 (page - 173).

13.2 Lung Injury Following Red Cell Transfusion

ALI following cardiac surgery is known to occur for a multitude of reasons. Only the most severe forms have been described in relation to RBC transfusion. Difficulty has existed with the diagnosis of subtler forms of the condition in the past as the various markers measured are non-specific for the degree of pulmonary endothelial damage[74;151]. Exhaled NO analysis offers the possibility of addressing this issue[152–154]. Although our results demonstrated no significant associations this could reasonably be attributed to the limited nature of the study.

13.3 Improving Anti-coagulation Strategies for Cardio-pulmonary Bypass

ACT based heparin management offers the advantage it is a simple, safe and cost effective technique[158;160;161]. However, several concerns have been raised over its accuracy during CPB. Conditions of hypothermia and haemodilution, as encountered during CPB, may lead it to under estimate the requirement for heparin[162–164]. This has led to the advocacy of HMSs by some investigators[164;166–169]. Our results suggest that the use of ACT may confer similar benefits provided additional heparin is given during CPB, independent of the measured ACT.

Table (13.1) *Suggested considerations for RBC transfusion from 0-48 post-operatively. RBC = red blood cell, RCV = red cell volume, BSA = body surface area, Hb = haemoglobin concentration, CPB = cardiopulmonary bypass and SVO_2 = mixed venous oxygen saturation.*

Small initial RCV	↑ Age
	Female Gender
	↓ Height/ Weight / BSA
	↓ Hb
Excessive RCV Loss	Surgical Bleeding
	Coagulopathy - ↑ CPB Time / Hypothermia
Haemodilution	↑ CPB Time
	↑ Clear Fluid Volume
Inadequate Circulation / Poor Oxygenation	Poor Cardiac Function[a]
	Relative Hypotension [b]
	$SVO_2 < 50\%$
	Myocardial Ischaemia [c]
	Sepsis
	Hyper-metabolism

[a] Ejection fraction $< 30\%$.

[b] Mean arterial pressure 70-80% of baseline or 60mmHg. This may be higher in patients with severe hypertension.

[c] New ECG ST segment depression > 0.1mv or elevation > 0.2mv non indicative of pericarditis.

Chapter 14

Future Research

14.1 Establishing the Impact of a Red Cell Volume Guideline on Morbidity and Mortality

As the study in chapter 9 (page - 106) was designed to detect a significant difference in RBC transfusion, it was underpowered to detect a significant difference in the clinical outcome measures recorded. What was gained however, was the knowledge that such a system may be feasible for wider clinical application. By recruiting larger, appropriately powered and blinded numbers then the full impact of an RCV based guideline on morbidity and mortality can be examined.

As discussed in section 1.4.3 (page - 21), no large prospective analysis currently exist examining the impact of a restrictive transfusion policy on survival following cardiac surgery. Thus, calculating the patient numbers required for such an analysis is difficult. If short term mortality (30d) is considered the primary outcome measure then taking the UK average of approximately 2% for elective cases may give a rough estimation of the required cohort size. A sample of 1798 would give 80% power to detect a 2% difference in 30d survival. Conducting such a study would be a major undertaking requiring a collaborative approach by a minimum of 5 centres. This would however, be the first large prospective evaluation of the effects of RBC transfusion on survival following cardiac surgery.

Summary of recommendations for future study;

- 30d mortality as primary outcome measure.
- Sample size of 1798 patients (899 per limb).
- Collaborative approach with a minimum of 5 centres to ensure adequate numbers.
- Funding secured to provide a research coordinator in each centre with central administration and collation of results.

14.2 Sodium Fluorescein Based Measurement of Red Cell Volume

Although the results gained in chapter 6 (page - 77), indicated that the NaF-RBC complex was not sufficiently stable for practical use, as described, there may still be the possibility of correcting for this deficiency. Performing further in vitro experimentation with the rate of decay of the RBC fluorescence may alow for the mathematical correction of the results obtained.

In figure 6.1, the error bar decay plot is given for the mean value of the four run samples analysed. Provided a gap of less than 60m exists between injectate preparation, blood sample aspiration and subsequent analysis then mathematical correction may allow for a reasonably accurate calculation of circulating RCV as per equation A.6.

Practically this would involve the following;

1. Recording of the injectate preparation completion time as t = 0.
2. Adjusting the fluorescence of the injectate at the time of patient injection as per the exponential decay curve (t = 1).
3. Adjusting the fluorescence of the analysed RBC's, as per the exponential decay curve, to correspond with the time of aspiration (t = 2).

Using such a methodology should allow the practical assessment as per the PIHD technique outlined in section 6.3.2 (page - 79). The same sample size calculation for the original proposed study could reasonably be applied (6.3.3, page - 80).

Summary of recommendations for future study;

- Multiple in vitro run analysis to accurately document exponential fluorescence decay curve.
- Sample size of 30 patients as previously described.
- Adjustment of fluorescence as per time point.
- Evaluation of NaF flow cytometry by PIHD.

14.3 The Association Between Lung Injury and Red Cell Transfusion

Although the pilot investigation in chapter 10 (page - 122) produced little in the way of tangible results, the results gained can be used to power a future investigation. It is also evident that several study adaptations would be required to improve the low completion rate and appropriately test post-operative lung function.

On determining sample size for a future investigation, it was calculated that a cohort of 168 patients would have 80% power to detect a difference of 10% in exhaled NO between the two groups. To improve completion rate, changes to the exhalation rate would be required. Approximately one third of patients in the initial investigation were unable to complete the 10s exhalation prescribed. The Nioxmino allows this to be reduced to 6s by use of a paediatric card. This was not done with the pilot investigation as the different times do not compare like with like.

A further weakness of the study construct in chapter 10 (page - 122) was that no measurements of post-operative lung function were made. This could be rectified by performing repeat PFT measurements at 48h post-operatively and possibly at 5d/pre-discharge. Results could be further improved by the measurement of carbon monoxide (CM) transfer factor[218].

Summary of recommendations for future study;

- % Reduction in exhaled NO at 48h as primary outcome measure.
- Sample size of 168 patients (84 per limb).
- 6s exhalation for NO measurement prior to surgery and at 48h following operation.
- PFTs recorded prior to surgery and at 48h and 5d post-operatively. To include CM transfer factor.

14.4 Continuous Heparin Infusions for Cardio-pulmonary Bypass

As discussed in section 12.9 (page - 165), the results obtained in chapter 11 (page - 132) suggest that additional heparin administration during CPB is required for coagulation system preservation. This can be achieved by the use of HMSs or by the administration of heparin independent of ACT.

The ACT system employed in our study gave additional heparin as per CPB crystalloid administration. As such a system is intermittent and unpredictable in nature, PH_{Conc} is not maintained at a steady concentration. It may be expected that maintaining a constant PH_{Conc} will result in even greater levels of coagulation preservation.

This hypothesis may be examined by the use of continuous heparin infusions during CPB. The relative efficacy of such a technique in maintaining PH_{Conc} has been documented previously when compared to the use of an HMS[219]. One approach to such an investigation would be the use of three study limbs with varying techniques for additional heparin administration;

1. Heparin/protamine as per standard ACT based nomogram.
2. Heparin/protamine as per standard ACT based nomogram with continuous heparin infusion.
3. Heparin/protamine as per standard ACT based nomogram with continuous heparin infusion and additional dosage as per supplemental fluid.

On determining sample size for such a study, it was calculated that a cohort of 84 patients would have 80% power to detect a difference of 10mg/l in TAT between the three groups.

Summary of recommendations for future study;

- Post-CPB change in serum TAT complex as primary outcome measure.
- Sample size of 84 patients (28 per limb).
- Heparin/protamine dosage as per standard ACT based nomogram with varying strategies for additional heparin.

Part IV

Appendices and References

Appendix A

Red Cell Volume Calculations

The basis for all RCV calculations is BSA. In all instances the formula used was that recommended by Gehan and George (equation A.1)[170];

$$S = 0.0235xHt^{0.42246}xWt^{0.51456} \tag{A.1}$$

Where; S = body surface area (m^2), Exp = exponential, Ht = height (cm) and Wt = weight (kg)

The most robust formulae for the calculation of RCV have been provided by the ICSH[171]. Seperate formulae have been provided for males (equation A.2) and females (equation A.3);

$$RCV = (1486xS) - 825 \tag{A.2}$$

$$RCV = (1.06xA) + (822 + S) \tag{A.3}$$

Where; RCV = red cell volume (ml), A = age (y) and S = body surface area (m^2).

Once RCV has been calculated both BV (equation A.4) and PV (equation A.5) can be derived using patient Hct;

$$BV = 100xRCV/Hct \tag{A.4}$$

$$PV = BV - RCV \tag{A.5}$$

Where; BV = blood volume (ml), RCV = red cell volume (ml), Hct = haematocrit (%) and BV = blood volume (ml).

When performing NaF flow cytometry, RCV can be calculated according to the following equation A.6;

$$RCV = (RBC_i x V_i x Hct_{ven})/(RBC_p x F) \tag{A.6}$$

Where; RCV = red cell volume (ml), RBC_i = RBC count per ml of the injectate, V_i = volume of the injectate (ml), Hct_{ven} = venous haematocrit (%), RBC_p = RBC count per ml of venous blood and F = fraction of fluorescent to non-fluorescent RBC's.

We chose PIHD to assess the above technique. The volume of RBCs removed in the PIHD bag was to be calculated as follows (equation A.7);

$$RCV = (PIHD_{wt} x Hct_{bag})/SG_{wb} \tag{A.7}$$

Where; $PIHD_{wt}$ = weight of PIHD blood (g), Hct_{bag} = Hct (%) of PIHD blood and SG_{wb} = specific gravity of whole blood.

Appendix B

Oxygen Calculations

Oxygen may be transported bound to [Hb] or contained in solution as represented below (equation B.1). This formula to calculate the oxygen content of blood applies to both the arterial (arterial oxygen content (CaO_2)) and venous (venous oxygen content (CvO_2)) circulation.

$$ConO_2 = (1.36x(SO_2/100)xHb) + (PaO_2xZO_2) \tag{B.1}$$

Where; $ConO_2$ = the oxygen content of blood (ml/dl), 1.36 = ml of oxygen bound per g of [Hb], SO_2 = oxygen saturation (%), PO_2 = oxygen partial pressure (mmHg) and ZO_2 = the solubility coefficient of oxygen in blood for a given temperature (ml O_2 per dl of plasma per mmHg).

Under normal environmental conditions the oxygen content in solution may be considered negligible (<2%). As outlined above, it is calculated by the product of the arterial partial pressure of oxygen (PaO_2) and the solubility coefficient for oxygen in blood (ZO_2). The ZO_2 may be calculated as follows (equation B.2)[98;220];

$$ZO_2 = [(4xHb + 346)xT + (130xHb + 35,000)]x0.0001/760 \tag{B.2}$$

Where; ZO_2 = solubility coefficient (ml O2 per dl of plasma per mmHg) and T = temperature (^{0}C).

DO_2 (ml/min/m^2) is considered a product of the CaO_2 and an individuals cardiac CI (l/min/m^2) at the time of sampling (equation B.3);

$$DO_2 = 10xCaO_2xCI \tag{B.3}$$

Where DO_2 = oxygen delivery (ml/min/m^2), CaO_2 = arterial oxygen content (ml/dl) and CI = cardiac index (l/min/m^2).

When considering estimates of DO_{2Crit}, a correction factor is required to allow for hypothermia (equation B.4);

$$Coef = exp^{[(0.08329xT+1.5234)/100]} \tag{B.4}$$

Where; Coef = correction coefficient, exp = exponential and T = temperature (^{0}C).

VO_2 (ml/min/m^2) may be represented mathematically as the difference in CaO_2 and CvO_2 multiplied by the CI (equation B.5);

$$VO_2 = (CaO_2 - CvO_2)xCIx10 \tag{B.5}$$

Where; VO_2 = systemic oxygen uptake, CaO_2 = arterial oxygen content (ml/dl), CvO_2 = venous oxygen content (ml/dl) and CI = cardiac index (l/min/m^2).

QO_2 (%) is the proportion of systemic oxygen delivered that is actually utilised by the tissues (equation B.6);

$$QO_2 = (VO_2/DO_2)x100 \tag{B.6}$$

Where; QO_2 = oxygen extraction (%), VO_2 = oxygen consumption (ml/min/m^2) and DO_2 = oxygen delivery (ml/min/m^2).

Appendix C

Red Cell Volume Guideline Derivation

As described briefly in chapter 9 (page - 106), the data obtained in chapter 5 (page - 66) was used to design the RCV based transfusion guideline. This process was completed stepwise as follows (tables C.1 and C.2);

1. Data was divided into males (n = 18) and females (n = 12).

2. For each gender, the data was divided roughly into 3 weight ranges ($\leq$ 77, 78 - 97, $\geq$ 98 for males. $\leq$ 54, 55 - 74, $\geq$ 75 for females).

3. For each gender and weight based subdivision the mean pre-operative RCV was noted.

4. This was then multiplied by a theoretical threshold ratio, descending from 0.85 - 0.6, to give a RCV transfusion threshold. A variable threshold was incorporated as an estimate of the ability to tolerate RCV loss (section 12.2, page - 154).

5. Under normovolaemic conditions i.e. static pre-operative BV, the Hct this RCV threshold would represent was derived (equation C.1);

$$Hct_{Th} = (RCV_{Th}/BV_{Pre-op})x100 \tag{C.1}$$

   Where; Hct_{Th} = threshold haematocrit (%), RCV_{Th} = threshold red cell volume and BV_{Pre-op} = pre-operative blood volume (ml).

6. For this value to have relevance post-operatively it was necessary to include a blood volume expansion factor i.e. the ratio of pre-operative BV to post-operative BV. A factor of 1.17 for males and 1.26 for females was used*.

7. By substituting pre-operative BV with the post-operative value in the above formula (equation C.1), a meaningful RBC transfusion threshold was obtained (tables C.1) and C.2)†.

*No significant association was found between weight and BV expansion. Hence, only gender was used to discriminate when applying a BV expansion factor.

†The [Hb] threshold for the male 78 - 79kg subdivision was changed from 7.7g/dl to 7.8g/dl. This was to ensure the linearity of the transfusion model.

Table (C.1) *RCV algorithm derivation for males. Where; Wt = weight, Ind = induction, RCV = red cell volume, RCV_{Th} = red cell volume threshold, NDil = non-diluted, Hct_{Th} = haematocrit threshold and RBC_{Th} = red blood cell threshold.*

Wt	Ind RCV	RCV_{Th}	RCV_{Th}	NDil Hct_{Th}	RBC_{Th}	
(kg)	(ml)	(Ratio)	(ml)	(%)	**Hct (%)**	**Hb (g/dl)**
$\leq$ 77	1948	0.70	1363	29.4	**25.1**	**8.4**
78 - 97	2249	0.65	1462	27.3	**23.3**	**7.7**
$\geq$ 98	2619	0.60	1571	25.2	**21.5**	**7.2**

Table (C.2) *RCV algorithm derivation for females. Where; Wt = weight, Ind = induction, RCV = red cell volume, RCV_{Th} = red cell volume threshold, NDil = non-diluted, Hct_{Th} = haematocrit threshold and RBC_{Th} = red blood cell threshold.*

Wt	Ind RCV	RCV_{Th}	RCV_{Th}	NDil Hct_{Th}	RBC_{Th}	
(kg)	(ml)	(Ratio)	(ml)	(%)	**Hct (%)**	**Hb (g/dl)**
$\leq$ 54	1238	0.85	1052	32.3	**25.6**	**8.5**
55 - 74	1417	0.80	1134	30.4	**24.1**	**8.0**
$\geq$ 75	1699	0.75	1274	28.5	**22.6**	**7.5**

Appendix D

Anti-coagulation Management

D.1 Activated Clotting Time

When calculating additional heparin and protamine dosage in chapter 11 (page - 132) (ACT_{Grp}) the following formulae as recommended by Bull et al were used (equations D.1 and D.2)[157];

$$Hep_{Dose} = Hep_{Initial}/(ACT_{PostHep} - ACT_{PreHep})x(480 - ACT_{Current}) \quad \text{(D.1)}$$

Where; Hep_{Dose} = heparin dose required (IU), $Hep_{Initial}$ = initial heparin bolus (IU), $ACT_{PostHep}$ = activated clotting time post intial heparin bolus (s), ACT_{PreHep} = activated clotting time prior to intial heparin bolus (s) and $ACT_{Current}$ = current activated clotting time (s).

$$Prot_{Dose} = Hep_{Initial}/(ACT_{PostHep} - ACT_{PreHep})x(ACT_{Current} - ACT_{PreHep}) \quad \text{(D.2)}$$

Where; $Prot_{Dose}$ = protamine dose required (mg), $Hep_{Initial}$ = initial heparin bolus (IU), $ACT_{PostHep}$ = activated clotting time post intial heparin bolus (s), ACT_{PreHep} = activated clotting time prior to intial heparin bolus (s) and $ACT_{Current}$ = current activated clotting time (s).

As measurements of BH_{Conc} only were obtained using the Hepcon HMS, the following formula was used to calculate PH_{Conc} (equation D.3);

$$PH_{Con} = (BH_{Con}x100)/(100 - Hct) \quad \text{(D.3)}$$

Where; PH_{Con} = plasma heparin concentration (IU/ml), BH_{Con} = blood heparin concentration (IU/ml) and Hct = haematocrit (%).

D.2 Heparin Dose Response

The HDR test is essentially based around ACT measurement with the addition of monitoring the effects of varying heparin doses on clotting time. This provides an in vitro estimation of an individuals sensitivity to heparin and can also be used to calculate the heparin dose required to reach the desired target ACT. The HDR cartridge contains six channels with an equal amount of kaolin reagent. Channels 1 and 2 contain USP porcine heparin to reach a sample heparin concentration of 2.5u/ml once the blood has been dispensed. Channels 3 and 4 similarly contain heparin to reach a sample heparin concentration of 1.5u/ml while channels 5 and 6 do not contain heparin and are used to obtain a baseline ACT. The clotting time results from each channel are used to calculate the slope response to heparin and the resultant dose required for the desired ACT. The formulae that provide the basis for these calculations are given below (equations D.4, D.5, D.6 and D.7)[221].

$$Slope = 1/2(Slope1 + Slope2) \tag{D.4}$$

$$Slope_1 = C - B/I, Slope_2 = B - A/H \tag{D.5}$$

Slope is given in ACT prolongation in seconds (s) per unit (u) of heparin (USP) per ml of BV*. Where; A = average clotting time of channels 5 and 6 (s), B = average clotting time of channels 3 and 4 (s), C = average clotting time of channels 1 and 2 (s), H = 1.5u/ml (channels 3 and 4 channels 5 and 6) and I = 1.0u/ml (channels 1 and 2 channels 3 and 4).

*All heparin concentrations described here are given in USP units (u). To convert to IU (as used for dosage in chapter 11) a correction factor of 0.88 is required.

Once the slope is known the target heparin concentration can be calculated as follows (equation D.6);

$$Heparin(u/ml) = (T - A)/S \tag{D.6}$$

Where; A = average clotting time of channels 5 and 6, T = ACT target time (s) and S = slope (s/u/ml).

The final HDR equation allows the calculation of the required patient heparin bolus (equation D.7);

$$PatientBolusDose(u) = [RHx(BV + PV)] - PH \tag{D.7}$$

Where; RH = target heparin concentration (u/ml), BV = blood volume (ml), PV = pump volume (ml) and PH = pump heparin (u).

D.3 Heparin Protamine Titration

The HPT cartridges contain thromboplastin and protamine of varying concentration in each channel. The channel that most accurately neutralises the contained heparin will clot first in the test. Where the measured heparin concentration is below the target value the additional dose required is calculated as follows (equation D.8)[221];

$$HeparinRequired(u) = Vx(RH - MH) \tag{D.8}$$

Where; RH = target heparin concentration (u/ml), MH = measured heparin (u/ml) and V = volume (patient, pump, or total) (ml).

A similar principle is used to calculate the protamine dose required to neutralise all the circulating heparin at the termination of CPB (equation D.9);

$$ProtamineDose(u) = (MHxV)xP : H \tag{D.9}$$

Where; MH = measured heparin (u/ml), V = volume (patient, pump, or total) (ml) and P:H = protamine heparin ratio.

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